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INVESTIGATIONS IN GREAT BRITAIN OF FURUNCULOSIS OF THE SALMONIDAE

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ATTENTION in Great Britain was first directed to furunculosis among fresh-water fish by a serious outbreak of the disease in certain rivers of the south-west of England in 1911. In all probability, however, the disease was present, although not definitely recognised, for some years before that date. On the continent of Europe it occurred in fish farms at least as early as 1890 and was described and studied by Emmerich and Weibel in 1894.

The disease continued in England sporadically during the years following 1911. The outbreaks among salmon in the south-west were not so serious as in 1911 but the disease manifested itself and continued in certain trout streams. In 1914 it appeared in a limited area in Ireland. In 1926 a considerable mortality among salmon in two rivers in Scotland was traced to the disease, and it was again observed in Scotland in 1927. By 1928 it had spread to thirteen additional rivers and was causing serious mortality in commercially important salmon fisheries.

The outbreak in 1911 was carefully investigated and reported on by Masterman and Arkwright, but in 1928 a further investigation on a larger scale was deemed to be necessary. This work, which was commenced as an unofficial research, was handed over in 1929 to the formal charge of a departmental committee* composed of scientific workers and persons interested in fisheries. The committee were fortunate in having as one of their number, Sir Joseph Arkwright, who had personally studied the disease in 1911. The committee were instructed to investigate the disease and to recommend measures for its control. Recommendations were made and, as

*The writers of this review were members of this committee and one of them (T.J.M.) was Chairman.

will be seen later, these recommendations ultimately formed the basis for a Bill which has now been passed into law.

At the commencement of their investigations the committee satisfied themselves as to the nature of the disease, and the specific ætiological relationshp of *Bacillus salmonicida* (Emmerich and Weibel) to furunculosis was fully confirmed.

Introduction and Spread of Furunculosis in Great Britain.

The circumstantial evidence, and none other was available, all pointed to the fact that the disease originated on the Continent from fish, notably trout, in fish farms: it was apparently imported with live trout transferred from continental to British fish farms and thence was introduced into the streams and rivers of the country by stocking. There can be no doubt that at first the disease was not recognised and its potential danger was certainly not appreciated either by trout farmers or by others interested in the British fisheries. No record was therefore kept of its occurrence in either farms or rivers. The committee's inquiries, however, showed that the rivers in which the disease appeared were for the most part grouped round certain fish farms, some of which were definitely known to be infected. Some striking cases of outbreaks of the disease following on the introduction of trout from farms were reported and confirmed. In the course of their investigations the committee were able to confirm through personal inquiries by one of their number that the disease still existed in practically all of the fish farms of at least one country in Europe from which large numbers of trout are exported alive.

Scientific Investigations conducted by the Furunculosis Committee.

In addition to tracing the source and spread of the disease in Great Britain, the committee organised and conducted a complete investigation of the disease, commencing with the biological characters, identification and pathogenesis of the causative organism, and proceeding through various stages to the experimental study of the mode of spread of, and the causes predisposing to, an active infection. Concurrently with the later experiments in the laboratory large scale experiments were undertaken in open ponds and in a large concrete tank in order to study the epizootiology of the disease and the method of continuance of the infection from year to year in the same water. Finally attention was given to feasible methods of controlling the disease and observations and experiments were also conducted on the bacteriophage for *Bacillus salmonicida*.

Occurrence and Distribution of Furunculosis in England and Scotland.

From the time of its first appearance in England the disease has been enzootic in certain of the chalk streams of the south in which valuable trout fisheries are maintained by continuous stocking. But apart from these streams and the original area of infection in the

south-west of England, the disease was reported in 1926 among salmon in four districts. By 1934 the number of districts in which salmon or trout or both were infected increased to thirty-one.

The disease was not reported in Scotland until 1926, possibly because trout fisheries of the southern English type do not exist; stocking from fish farms was relatively light and very largely confined to lochs and reservoirs. It is very probable also that foreign trout were brought to Scottish fish farms at a much later date than to the English establishments. The disease was first seen among salmon in three rivers. The year 1927 being cold and wet was not favourable for the development of the disease, but in 1928 it was proved to exist in no less than fifteen streams. The number gradually increased to twenty-eight in 1934 and now the total is probably over thirty.

It was chiefly the loss to commercially important salmon fisheries which justified the work undertaken by the committee. It is no exaggeration to say that in suitable circumstances the salmon population of a particular area in a river may be decimated: in certain places large numbers, often hundreds, of salmon were picked up dead, and many others lay dead out of reach or out of sight in pools and under rocks. The loss was the more serious in that it invariably occurred above the netted areas and therefore among fish which, except for the slight loss due to angling, would all form part of the spawning stock.

At first outbreaks among salmon led anglers and others to the opinion that the disease was brought in from the sea. Considerable investigation was devoted to this aspect of the problem and ultimately it was found that when salmon fresh from the sea, coming up the river on a flood, are gathered together in pools they rapidly fall victims to the infection disseminated from the local brown trout population, some of which are carrying the specific micro-organism.

Environmental Factors Favouring the Occurrence of Furunculosis.

After enquiry it became abundantly clear that certain conditions are necessary for the production of an epizootic among salmon. The primary factor is the presence of the causative parasite for which as stated the local brown trout population also serve as hosts. A second factor is the aggregation of salmon in relatively large numbers in a comparatively limited space such as a pool below a fall or in isolated pools in long stretches of otherwise shallow water. Other essentials are a low river level and a water temperature between 55° and 66° F. (approximately). Moreover, salmon fresh from the sea are apparently less resistant to the disease than others which have been some time in fresh water. An epizootic among brown trout is brought about by similar conditions, *e.g.*, a heavy stock, absence of floods, and a favourable water temperature. Conditions of overcrowding normally exist in fish farms and, if the infection be present,

an epizootic may develop at any time when the temperature is within the optimum range, although those in charge learn to foretell the danger period and take such steps as are practicable (*e.g.*, by increasing the water supply) to limit the effects of the disease. Actual details of the manifestations and occurrence of the disease in fish farms have been difficult to obtain owing to commercial interests. Its presence in many farms was, however, reported, and in certain cases the persistence of the infection from year to year with accompanying annual loss among the fish older than one year was admitted. In one outbreak, for instance, a loss of 15 per cent. of a valuable stock of large trout kept for spawning was said to have occurred in one year. In addition to the normal occurrence of the disease among trout in the summer months a secondary outbreak may also occur in the winter and early spring months, after the spawning period when the water is spring fed and has a temperature of from 42° to 46° F. No doubt the much lowered vitality of the fish at this season permits a greater aggressiveness of *Bacillus salmonicida,* even below its optimum temperature for vegetative growth. A similar happening has been observed among kelt salmon in at least one river when the water temperature has been about the level mentioned above.

Distribution of Furunculosis among Fresh Water Fish.

Members of the *Salmonidæ* which enter, or live in, fresh water are all subject to the disease although some species are more susceptible than others. All appear to be highly resistant in their youngest stages (one year and under). Salmon (*Salmo salar*), and trout (*Salmo trutta*) of both the migratory and non-migratory race, are very susceptible, as are also brook trout (*Salvelinus fontinalis*). Rainbow trout (*Salmo irideus*) and grayling (*Thymallus vulgaris*) are apparently less susceptible. The Coregonoid fishes are all reported to be subject to the disease. Various fresh water fishes not belonging to the *Salmonidæ* are also susceptible and some (*e.g.*, tench, carp, catfish, bullhead, pike) have been reported as suffering from the disease in natural surroundings. There is no evidence that eels in Great Britain are affected by furunculosis. This question will be referred to later in connection with the experimental observations.

Bacillus Salmonicida and its Relationship to Furunculosis.

The causative relationship of *B. salmonicida* to furunculosis of the *Salmonidæ* has rarely been questioned. Koch's postulates are fully confirmed by this organism: it is constantly present in the lesions, it can be cultivated from them artificially and cultures inoculated into healthy fish reproduce the typical disease. It was suggested, however, to the Furunculosis Committee that a filterable virus might be the causal agent, and that *B. salmonicida* had only a concomitant or secondary rôle. This idea was based on the nature of the lesions (referred to later) which are somewhat dissimilar to those of bacterial infections in the higher animals. Experiments designed to

demonstrate a filterable virus, if such should be present, in the disease yielded quite negative results. Only unfiltered pathological material containing *B. salmonicida* was able to reproduce the disease under experimental conditions ; bacteria-free filtrates were inactive.

B. salmonicida is highly virulent on inoculation of cultures in trout, gold-fish, minnows and frogs, and both inoculation experiments and observations of the natural disease have shown clearly that the condition is essentially a general infection. The boil-like lesions on the surface (which give the disease its name) are quite inconstant in their occurrence and are more a feature of the subacute form of the disease, being absent in the rapidly fatal cases. These lesions are foci of tissue necrosis and are devoid as a rule of the vascular or tissue reactions which are characteristic of bacterial disease in the higher animals. Other notable features of the disease are necrosis and liquefaction of the kidneys and spleen, and in all the lesions the specific organism is demonstrable in large numbers and is likewise present in the circulating blood. Studies of the disease in Great Britain have shown that the pathological features of the disease are most variable and that fish may die of the infection without any characteristic appearances. This emphasises the necessity for diagnosis by bacteriological examination and since 1928 an official diagnostic service has been in operation in Great Britain, first instituted by the Furunculosis Committee and later maintained under the Diseases of Fish Act. Bacteriological observations have also brought to light the fact that the infection may occur in a latent form and that apparently healthy fish may carry the specific organism for considerable periods. This will be referred to again later.

Biological Characters and Identification of Bacillus Salmonicida.

It is unnecessary to discuss here the biological characters of *Bacillus salmonicida,* but the question has arisen from time to time whether it can easily be identified and whether other bacteria may be confused with it. Its characteristic brown pigment produced in culture is undoubtedly an important feature and has been relied on, along with other characters, in the identification of the organism. Of course the degree of pigment production is somewhat variable and there is evidence that this property may be inhibited when the organism is growing in mixed culture with other bacteria, as in cultivation tests of fish whose carcases are contaminated with putrefactive bacteria. Exceptionally, chromogenic bacteria have been isolated from water and fish which superficially resemble *B. salmonicida,* but careful examination of these has always revealed clear differences and the experienced laboratory worker has found little difficulty in the bacteriological diagnosis of furunculosis. On the other hand it has always been considered desirable to have confirmatory criteria of the identity of *B. salmonicida* ; the inoculation test in trout or gold-fish and the complement-fixation test with a

specific antiserum have supplied such criteria and have proved valuable where any doubt has arisen regarding other biological features. While *B. salmonicida* like other bacterial species is subject to some degree of variability, strains, including those from different areas and different countries, have proved remarkably uniform in characters. From the more practical standpoint the following questions have arisen: whether this organism is an obligate parasite, what viability it possesses apart from its host, and within what temperature range it can grow. These questions were very carefully investigated by Williamson (1928) working under the Furunculosis Committee. She found that in waters of various types the organism could only survive for a few days at most, and all her observations pointed to the fact that *B. salmonicida* is a strictly parasitic species and cannot exist as a saprophyte under natural conditions. In very extensive bacteriological observations on water, fish, fresh-water invertebrates, etc., *B. salmonicida* has never been isolated apart from furunculosis, with the exception of the carrier fish referred to above. Williamson found that the temperature range for growth is approximately 41° to 90° F. and the optimum about 68° F. *under laboratory conditions.*

Experimental Observations on Furunculosis.

Experimental observations have thrown much light on the nature of the disease and its transmission; and it has been found easily possible to carry out both small- and large-scale inoculation and infection experiments on brown trout kept in laboratory tanks (of varying size) either with running or aerated water. For inoculating the fish a technique of anæsthetising them in water containing urethane has been devised and has proved most helpful in this work. When large doses of cultures of *B. salmonicida* are injected parenterally by various routes a rapidly fatal infection results, the fish dying without characteristic focal lesion, *e.g.*, boils; when the dose is reduced, however, it is then found possible to reproduce the typical disease with all its known features. The lethal dose of a recently isolated *B. salmonicida* culture may be exceedingly small and the organism possesses a quantitatively high virulence. Infection can also be brought about by exposure of a fish to water containing the organism and by feeding with cultures.

Spread of Infection; and Carriers of Bacillus Salmonicida.

The occurrence of healthy carriers of *B. salmonicida* seemed to the Furunculosis Committee one of the most important aspects of the whole epizootiological problem and special attention was devoted to it. It has been shown that such carriers occur among the stocks of fish farms and in this way apparently healthy stock may spread the disease to natural waters. Of course, under ordinary conditions carriers can only be recognised by taking a sufficient sample of the stock, killing the fish and making cultures from the carcases.

Carriers have also been found among the natural stock of rivers. The site of harbourage of *B. salmonicida* is the kidney and it is usually sufficient to make cultures only from this organ. Carriers of *B. salmonicida* are of the " incubationary " type and sooner or later die of the disease ; on the other hand they may remain healthy for several months.

Infection is spread from diseased fish which discharge the specific bacterium in large numbers into the water from the surface lesions and in their excreta ; and likewise healthy carriers contaminate the water from their excreta. The water is undoubtedly the medium through which the organism is transmitted and from which it invades the tissues of a new host through the gills, the alimentary tract or in some cases through a surface injury. Experimentally it has been shown that the infection can be transmitted through water from fish to fish without actual contact. There has been no evidence that the infection is congenital and conveyed by ova, though possibly external contamination of ova from an infected parent might spread the disease in a hatchery. It should be emphasised that the carcase of an infected fish decomposing in a water may liberate large numbers of the specific organism and so constitute for a time a continuing source of the infection.

Experiments on the Factors which determine the Occurrence of the Disease.

As regards the immediate factors which determine the development of the active disease, experiments with trout in tanks containing water at varying temperatures have shown that the optimum water temperature is from 59° to 64° F., and this finding agrees approximately with field observations which indicate that under natural conditions the optimum is from 55° to 66° F. The question arises whether this optimum depends on the optimum for the growth of the causative organism. The optimum for growth *under laboratory conditions* is somewhat higher, and other factors, influenced possibly by temperature, may come into play, such as the resistance of the host to the parasite. On the other hand it can be understood how a low temperature by inhibiting the growth of the organism would also arrest the disease. Experiments with trout at different ages have revealed remarkable differences in susceptibility ; thus fry are highly resistant ; trout under two years are more resistant than older fish. Experiments with different species of *Salmo* have also elicited differences in susceptibility. Thus, *S. fario* is more susceptible than *S. irideus,* and *S. fontinalis* occupies an intermediate position. Even within the same species, however, individuals vary greatly in susceptibility.

It may be said here as regards the infection in fish other than the *Salmonidæ* that eels possess a high resistance to this infection, and there is no evidence that they contract the disease under natural conditions.

Large Scale Experiments on Furunculosis.

Much information on the natural history and epizootiology of the disease has been obtained by large scale experiments with colonies of brown trout and other *Salmonidæ* kept in ponds and large tanks. It has been found possible to start an epizootic in such colonies by inoculating a few trout with *B. salmonicida* culture and then introducing them into the pond or tank. These experiments, of course, have been carried out during the summer season when the water temperature was at or near the optimum. In the pond experiments the results have indicated that such epizootics can only be readily induced when the fish are relatively crowded, *e.g.*, one fish to 6 to 20 c. ft. of water. This is of special interest, as field observations have shown how low water and overcrowding of the fish, *e.g.*, in pools, increase the prevalence of furunculosis. These large scale experiments have also shown how fish may become infected within a few hours in a water containing diseased individuals and die even within three days. In such epizootics, however, the actual mortality among brown trout may not exceed 35 per cent. On the other hand grilse and sea trout may show a mortality of 86 to 100 per cent., and these fish when introduced into an infected colony of brown trout may die in three or four days. This explains how salmon entering an infected river from the sea may so rapidly contract the disease and die from it that they convey almost the appearance of having brought the disease with them from the sea. Finnock are much less susceptible than adult sea trout.

In the epizootics referred to, apart from the fish which develop the active disease others become infected but remain healthy and thus become carriers ; these, however, die at varying intervals afterwards. It would appear that when the infection is introduced into a colony of brown trout under favourable conditions the spread of the infection is very rapid and extensive at an early stage ; some fish die from the peracute disease ; others suffer from a subacute form ; others again, as stated, resist the infection for a variable time, thus becoming carriers. In this way the epizootic rapidly attains a peak and then slowly declines, fish dying intermittently over a fairly long period afterwards. If fresh batches of fish are added to the infected colony the epizootic is renewed and thus the active epizootic disease can be maintained by repeated addition of healthy fish. As stated above, these experiments have been carried out during the summer and it has been striking how in the autumn when the temperature has fallen, *e.g.*, to 50° F., the disease has ceased. Carriers, however, have persisted in the colony and in one experiment in a large tank in which survivors from the summer epizootic were kept over the winter till the following summer an epizootic was induced by the addition of a fresh batch of trout. (It may be said that the presence of the infection in the added batch was excluded.) This clearly indicated the persistence of carriers during the winter from one summer to another. Probably in this way the infection is main-

tained in a river during the cold season and recrudesces under favourable conditions in the following summer.

Bacillus Salmonicida Bacteriophage.

A bacteriophage capable of destroying *B. salmonicida* by lysis was demonstrated by Todd (1933) in the waters of certain English rivers and was also described in the Second Interim and Final Reports of the Furunculosis Committee (1933 and 1935). The questions arose whether its presence in water might be an indication of the occurrence of the infection among fish and whether bacterioplage might be practically applied in the control of the disease. The Furunculosis Committee were unable to accept this bacteriophage as an exact indicator of the disease. While a bateriophage may possess the power of killing the appropriate bacterium in laboratory experiments, as is well known this property may not be exerted in the animal body ; moreover the action of a bacteriophage on a bacterial growth does not necessarily connote the destruction of all individuals ; some may prove resistant and continue to flourish even in the presence of the phage. It seemed to the Committee that in any case this bacteriophage could have little or no application for the control of the disease in natural waters.

Since the Committee completed their investigations under their official instructions, some research work has been carried out on *B. salmonicida* bacteriophage in the Bacteriology Department of Edinburgh University with the aid of grants from the Development Commissioners. The two questions referred to above have been carefully studied. The occurrence of the phage in rivers has been extensively investigated and it has been found to be very widely distributed. Its presence or absence cannot be correlated with the presence or absence of the disease, and it has been found in river waters even during the winter. It has also been isolated from city sewage. It has been found in cultures of *B. salmonicida* just isolated from fish dying of the acute disease. This phage is relatively specific for *B. salmonicida* and few other organisms isolated from water have been found susceptible to it and none have been found to "carry" it. Its actual origin, therefore, in a water from which *B. salmonicida* infection is absent is still obscure. (It should be mentioned that its demonstration in water is effected by an "enrichment" method in which *B. salmonicida* culture is added to a large quantity of water, the mixture being incubated for 24 to 48 hours and then filtered, and the filtrate tested for phage action by appropriate methods. The cultures used for enriching are of course controlled to ensure that they do not primarily carry the phage).

The influence of this phage on an epizootic of furunculosis has also been tested experimentally. Epizootics have been set up in colonies of about fifty trout in large tanks and strong bacteriophage preparations added to the water, the mortality being compared with that of similar control colonies. No prophylactic influence has been observed. Phage preparations have also been introduced by injec-

tion into fish along with *B. salmonicida* culture and while some delaying effect on the disease has been noted the phage has not protected against a fatal result. These observations do not offer any indication that the phage could be readily used for controlling the disease even in confined waters such as those of a fish farm.

Recommendation of the Furunculosis Committee; and the Diseases of Fish Act.

As a result of their investigations the Committee finally came to the conclusion that the disease was a source of considerable danger to the salmon and trout fisheries of Great Britain and that its continuation and spread not only gave cause for alarm but were also likely to be fostered in the absence of control. They were satisfied that voluntary effort could not be successful and they recommended legislative action with two main ends in view: (1) to stop the importation of further infection from the Continent; (2) to restrict the possibility of further spread of infection in Great Britain. It was proposed that the general principles of such legislation should be those adopted in controlling infectious disease in stock animals, along with the prohibition of the importation of live fish of the salmon family (a considerable trade in live trout for restaurant use and for re-stocking existed) and the control of other fish and fish eggs imported alive.

After some delay the Government accepted the recommendations of the Committee and introduced and passed the necessary legislation (Diseases of Fish Act, 1937). This Act came into force on November 1st, 1937, since when the importation of live fish of the *Salmonidæ* has been prohibited and the importation of their eggs and other live fish and fish eggs has been subject to licence. Furunculosis (and by Order other diseases also) has been made a notifiable disease, and powers have been taken by the Government to prohibit or control the movement of live fish, etc., from infected rivers or fish farms, and to inspect and to take any necessary samples from fish farms or other waters. It is as yet too early to state any results of the operation of the Act, but the three reports of the Committee and the preparation and passing of the consequent legislation have undoubtedly led to fish farmers giving much more attention to the disease and exercising much more care with their stock.

References.

First and Second Interim and Final Reports of the Furunculosis Committee, 1930, 1933 and 1935; H.M. Stationery Office, Edinburgh. (These contain references to other literature on the subject).

Acknowledgments.—The investigations of the Furunculosis Committee were aided by grants from the Development Fund. Most of the bacteriological work of these investigations was carried out by Dr. Isobel Blake (née Williamson) in the Bacteriology Department of Edinburgh University. The bacteriological and experimental observations of *B. salmonicida* bacteriophage were made by Drs. M. H. Christison and I. MacKenzie in the same department. (These have not yet been published.)

CLOSTRIDIUM WELCHII: NOTES ON THE RELATIONSHIP BETWEEN THE TYPES OF CULTURES AND THE PRODUCTION OF TOXIN

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Many workers in different parts of the world have studied the *Cl. welchii* group of organisms and its importance in the production of human infections and in the causation of certain diseases of animals, particularly those of sheep referred to as entero-toxæmias, has now been proved. Until 1923, however, when Gaiger and Dalling observed the association of *Cl. welchii* with lamb dysentery, the only organisms which had been intensively studied were those which had been isolated from human sources. A large number of these strains of human origin were isolated during the War from cases of gas gangrene resulting from wound infection with this organism, and although different generic names were used by French and German workers, *e.g.*, *B. perfrigens*, Fraenkel's gas bacillus, etc., they all appeared to correspond in their cultural characteristics and in their pathogenicity with the first strain of the species which was isolated and described by Welch and Nuttall in 1892.

This strain has been referred to as the "classical" strain of *Cl. welchii* and this description has been used to distinguish it and other similar strains of human origin from those isolated within recent years from certain animal sources. Its capacity to produce a potent toxin when grown under suitable conditions has long been recognised. Bull and Pritchett (1917) first demonstrated this fact and showed further that a specific antitoxin could be produced. Henry (1923) made an intensive study of the toxin and came to the conclusion that it could be divided into two fractions, a hæmotoxin capable of hæmolysing red blood corpuscles and a myotoxin which causes necrosis of muscle and skin and which is capable of being absorbed on to fresh muscle. He also showed that both the hæmo- and myotoxins were neutralised by the same antitoxin.

The variation in the toxin-producing powers of different strains has also been recognised, and McGaughey (1933), by selection and repeated sub-culture of colonies isolated two variants which, though resembling the present strain, varied in their capacity to produce toxin. One variant at one stage appeared to be incapable of producing any toxin but later was shown to produce small amounts, while the other variant produced toxin from three to six times as potent as did the parent strain.

The first indication that different types of *Cl. welchii* existed followed the observations of Dalling (1928). Working with strains isolated from cases of lamb dysentery, he showed that they produced a toxin which could be differentiated from that already described in the following important aspects :—

(1) The toxin was lethal to mice in comparatively small doses.

(2) The antitoxin of classical *Cl. welchii* strains did not neutralise this potent toxin while the antitoxin produced from lamb dysentery strains neutralised its own toxin and that of classical *Cl. welchii*.

(3) While Dalling, Glenny, Mason and O'Brien (1928) working with strains of classical *Cl. welchii* showed that there was a close relationship between the hæmolytic titre of the toxin and its toxicity, it was found that the hæmolytic power of this new toxin had no relationship to its toxicity and that the strains from lamb dysentery cases produced about 10 to 20 times less hæmolysin than did strains of classical *Cl. welchii*.

The next important observation was that of McEwen (1930) who described a *welchii*-like organism which he named *B. paludis*, isolated from sheep in the Romney Marsh district of Kent affected with a disease of the entero-toxæmia type, referred to locally as "struck." This organism produced a highly potent toxin and though there were apparently some minor cultural differences between *B. paludis* and the strain of *Cl. welchii* isolated from cases of lamb dysentery, Mason, Ross and Dalling (1931) considered, on the evidence of toxin-antitoxin neutralisation tests, that the two organisms were serologically identical. In the light of more recent observations, in all probability the toxin of the lamb dysentery strain used in these tests by these workers was deficient in a certain fraction which is produced by typical lamb dysentery strains.

Attention was now focused on still another variation in the group by the isolation in Western Australia by Bennetts (1932) from cases of entero-toxæmia in sheep of an organism which he named *B. ovitoxicus*. (A sub-culture of this organism and certain descriptive details were sent to us by Bennetts during 1930). In its morphological and cultural characteristics, this organism closely resembled the three varieties of *Cl. welchii* already recognised. It differed from them in that it produced a potent toxin which could only be neutralised by its specific antitoxin and not by any of the *Cl. welchii* antitoxin sera which were used by Bennetts in his preliminary investigations. This organism was considered to be the representative of a new species until some uncertainty arose as the result of an interesting observation made almost simultaneously by Gill, Bennetts and Montgomerie and Rowlands, *viz.*, that while certain batches of antitoxin prepared from a lamb dysentery strain completely neutralised the toxin of *B. ovitoxicus*, other batches prepared from the same strain failed to do so.

(Investigations referred to by Montgomerie and Dalling (1933) and by Dalling (1936) indicate that lamb dysentery strains are capable of producing several types of toxin and that apparently one strain had entirely lost the power of producing one type of toxin; this accounted for the differences in the antitoxin content of the different batches of serum used by the above observers.)

This observation indicated that there must be some relationship between the L.D. bacillus and *B. ovitoxicus* and that the latter represented a hitherto unrecognised type of *Cl. welchii*. A number

of strains which appeared to be identical with Bennett's *B. ovitoxicus* were isolated in close succession by workers in different parts of the world, thus Oxer (1932) in Tasmania and Gill (1932) in New Zealand each isolated the same organism from cases of pulpy-kidney disease in lambs. We also examined cultures isolated from lambs in this country, in America and in Greece and showed them to be similar to *B. ovitoxicus*.

During the period in which these different investigations were in progress, Wilsdon (1931) studied many strains of *Cl. welchii* procured from different sources and found that by a comparison of their toxin-antitoxin relationships they could be divided into four distinct serological types. Thus, type A comprised strains of classical *Cl. welchii*; type B, strains isolated from cases of lamb dysentery; type C, one strain of *B. paludis*, and type D, six strains isolated from lambs. No strains of *B. ovitoxicus* were available for comparison with these type D cultures, but these were procured later (Wilsdon, 1933) and were shown to belong to this type. He considered that the use of such terms as the "L.D. bacillus" and "*B. paludis*" should be discontinued as being unnecessary and apt to give rise to confusion. The following is a brief summary of his suggested system of classification :—

Type A strains produce type A toxin containing the W antigenic factor.

Type B strains produce type B toxin containing the W, Z and X antigenic factors.

Type C strains produce type C toxin containing the W and Z antigenic factors.

Type D strains produce type D toxin containing the W and X antigenic factors.

Glenny, Barr, Llewellyn Jones, Dalling and Ross (1933) studied the antitoxin contents of a number of sera prepared from *Cl. welchii* cultures. They confirmed Wilsdon's observations that such sera might contain one or more antitoxins according to the number of antigenic components in the immunising filtrates.

By using the guinea-pig intradermal and the hæmolytic methods of testing as well as the mouse intravenous method they showed that it was possible to detect a number of individual toxins in the filtrates of type B, C and D cultures. They distinguished between the different toxins by means of the Greek letters α (alpha), β (beta), γ (gamma), δ (delta), and ϵ (epsilon). A summary of their findings is given in the following table :—

Toxins of the Cl. welchii Group.

Culture Types.	*Toxins.*				
(Wilsdon)	α	β	γ	δ	ϵ
A	+	—	—	—	—
B	+	+	+	±	+
C	+	+	+	+	—
D	+	—	—	—	+

+ = toxin produced ; — = not produced ; ± = not invariably produced.

Wilsdon had inferred the presence of the majority of these toxins by the production of corresponding antitoxins in the sera of immunised animals but had been unable to demonstrate their presence in culture filtrates since he used only the mouse intravenous method of testing which measures the summation of several toxic effects.

It will be obvious from the above table that it is necessary to determine which of the toxins a member of the *Cl. welchii* is able to produce before concluding the type to which it should be assigned. The purpose of this paper is to draw attention to this point. We have carried out certain investigations in the laboratory in connection with the conditions necessary for the production of toxin by the four types of *Cl. welchii* and have shown that there are optimum conditions for the production of the different toxins and that unless these have been provided, a culture may be erroneously typed.

In connection with the nomenclature of toxins of the group, Prigge (1936) has shown recently that certain dried toxins prepared from type A culture filtrates may contain the two components described by Henry (1923) in varying proportions. He suggests that the term "Alpha-toxin" should be applied to the hæmolytic and "Zetatoxin" to the necrotic or lethal fraction.

Recognition of the Toxins.

Alpha (α) *toxin* can be readily recognised by its hæmolytic action on red blood cells, by its lethal effect on mice and by the necrotic reactions following its intradermal injection into guinea-pigs or rabbits.

The hæmolytic method is the most suitable for the detection of traces of toxin which are less than one mouse fatal dose or one reacting skin dose. Such traces of toxin will usually produce some evidence of hæmolysis of red cells. Following the intravenous injection of the toxin, within a period varying from a few minutes to several hours, according to the dose, the mouse appears ill, the coat becomes ruffled and the skin of the feet and mouth parts may become darker in colour. Death soon occurs. Some evidence of hæmaturia is almost invariably observed either before or after death.

Beta (β) *toxin* is not hæmolytic and can only be detected by its lethal action in mice and by the necrotic reaction following injection into the skin of guinea-pigs and rabbits. Traces of toxin which cannot be detected by direct methods can only be shown to be present by their action as a stimulus in the production of antitoxin.

Large doses of β toxin produce characteristic symptoms in mice following intravenous injection. They differ markedly from those produced by type A filtrates. When many fatal doses are injected the mouse twitches spasmodically and dies almost immediately. With smaller doses, although the latent period may be prolonged for some hours, the animal usually appears to be quite normal until the spasmodic symptoms appear following which death soon takes place.

Gamma (γ) *toxin.* Glenny *et al.* (1933) have pointed out that the presence of γ toxin can only be inferred by indirect methods of testing. It occurs in conjunction with β toxin in the filtrates of Type B and C cultures; it is lethal to mice but does not produce necrosis when injected intracutaneously into guinea-pigs. Although sera prepared from these cultures usually contain both β and γ antitoxins, instances have been recorded of sera which contained β but no γ antitoxin. Such sera failed to protect mice against filtrates containing β and γ toxins injected intravenously but prevented completely the occurrence of lesions in guinea-pigs injected intracutaneously.

Delta (δ) *toxin* should perhaps be termed a hæmolysin rather than a toxin since it can be most readily recognised by its hæmolytic action on red blood cells. It has not been possible to show by direct methods that δ toxin is lethal for animals or that it will produce necrosis when injected intracutaneously. Recent unpublished work of Glenny, Duff and Ross, however, suggests that δ toxin may be lethal to mice when injected intravenously.

Epsilon (ϵ) *toxin* is not hæmolytic and can only be detected by its lethal and necrotic action in mice, guinea-pigs and rabbits.

Large doses of the toxin injected intravenously into mice produce symptoms somewhat similar to those following the injection of β toxin. Mice injected with many fatal doses twitch spasmodically, twist and turn in a characteristic manner and there may be short spasms of rigidity when the animals appear to be dead.

This toxin differs from β toxin in that small doses or traces of toxin injected intravenously into mice will produce symptoms of paralysis after a latent period which may be as long as five days.

The symptoms produced by ϵ toxin may be complicated by the presence of α toxin if the filtrate is from an early growth of a type D culture and in this case symptoms of hæmaturia will almost certainly be found.

Since each of these toxins can only be neutralised by its specific antitoxin it is possible, by neutralisation tests, to determine which toxin or toxins are present in the filtrates of any one culture and by this means to ascertain the type of the culture. Such neutralisation tests, whether carried out by *in vivo* or *in vitro* methods, necessitate the use of sera which contain the specific antitoxins and although it has not been possible to prepare sera which contain either β, δ or ϵ antitoxins alone, we have found that by using sera prepared from type A, C and D cultures, it is possible to detect the presence of α, β, δ and ϵ toxins in filtrates; as a reference to the table on page 246 will show, these are the toxins which are significant for the classification of cultures. The sera which we have used in our work and which were prepared in horses by Mr. Glenny and his colleagues contained the following antitoxins :—

(1) Serum prepared from a type A culture contained α antitoxin only.

(2) Serum prepared from a type C culture contained a large proportion of β and δ antitoxins and a small proportion of α.

(3) Serum prepared from a type D culture contained a large proportion of ϵ antitoxin and a small proportion of α.

It has been observed that the sera of normal horses may contain antibodies to individual toxins. A further interesting point is the finding in the sera of animals immunised against specific toxins of antibodies to toxins not demonstrable in the material injected. Thus, for example, it is possible that the serum from an animal injected with the filtrate of a type D culture may contain α, β and ϵ antitoxins, the first and last only having been produced by active immunisation with the filtrate. It is only by referring to the unit content of the serum that it can be determined which antitoxins are present as the result of active immunisation and it is of the greatest importance to test sera prepared from type cultures both for their relative antitoxin content and for the presence of traces of antitoxins which are not normally produced by active immunisation.

The methods of testing which we have used for the qualitative examination of culture filtrates have been based on these described by Glenny *et al.* (1933) for the estimation of the unit values of α, β, δ and ϵ antitoxins. When testing for the presence of α or δ toxins by the hæmolytic method, mixtures of the filtrate and type A and C sera were made so that the total volume was 2 c.c. in each case. After standing for half-an-hour at room temperature, 0·5 c.c. of a 20 per cent. suspension of sheep red cells in saline was added to the mixtures which were then heated in a water bath at 37° C. for 1 hour. Readings were taken after 1 hour and again after 24 hours at room temperature.

When testing for the presence of β and ϵ toxins we have found that the mouse intravenous method of testing allows of a rapid diagnosis in the case of both these toxins. If the intradermal method is used a longer period must elapse before a diagnosis can be made, as neither β nor ϵ toxin produces its maximum necrotic reaction in less than 24 or 48 hours and during this interval the toxin may have deteriorated or become non-toxic.

In carrying out the tests, we have added each of the three type sera, undiluted, to volumes of the filtrate, also undiluted, in the proportion of approximately one part of serum to four of filtrate, so that the total volume of each mixture to be injected intravenously did not exceed 0·5 c.c.

As some filtrates contain both β and ϵ toxins we found it necessary to include mixtures containing both type C and D sera. The mixtures were allowed to stand at room temperature for half-an-hour before being injected into mice and the final results were recorded after the mice surviving the injection had been kept under observation for five days. When only small samples of culture growths were available, they were centrifuged and the clear supernatant fluids were used. This obviated the possible erroneous results attending the injection of many living organisms.

Factors Influencing the Production of the Various Toxins.

The factors influencing the toxin production of type A cultures have been studied by many workers and a number of methods have been described in detail for the preparation of α toxin.

We have found that by adopting one particular method, toxin of high value can be prepared in comparatively large volumes. For the preparation of the other toxins in their most potent form we have found that certain alterations of this method are necessary.

We have studied to some extent the optimum conditions for the production of each toxin and have found that measuring the strength or potency of the toxins in terms of units of antitoxin gives some indication as to whether the conditions provided have been the most suitable for the production of an individual toxin.

This point is of importance in connection with the classification of cultures where the ability to produce certain toxins determines the type to which an organism belongs. Although it is not necessary to estimate the potency it is essential to grow the organism under conditions which are known to lead to the production of each toxin in its most potent form.

Unless the organism has been given every opportunity to produce each of the toxins α, β, δ and ε, negative results are of little value and may lead to errors in typing.

For the production of the toxins we have used a medium having a base of meat infusion broth containing peptone. The minced meat (horse muscle) used in the preparation of the broth has been washed, partially dried and added to the broth prior to auto-claving.

We have had occasion to prepare considerable volumes of α toxin for use in the immunisation of horses and have found that the best results are obtained by adding a large proportion of the minced meat (approximately 50 per cent. by volume) to the broth and by harvesting after a short period of rapid growth at 37° C. After the point of maximum growth has been reached the value of the toxin usually deteriorates rapidly and if the incubation period is prolonged for two or more days it may no longer be possible to detect any α toxin in the culture filtrate.

In the case of β and δ toxins also, we have found that the most potent toxins are produced after a short period of rapid growth at 37° C. but that for both these toxins the additions of a large quantity of meat appears to be detrimental to the value and, as a rule, only 10 per cent. of meat by volume has been used.

For the preparation of ε toxin of high value better results have been obtained when a large proportion of meat (50 per cent. by volume) has been added to the broth and when the incubation period has been comparatively long. Bennetts (1932) observed that the toxicity of *B. ovitoxicus* cultures was greater after three days than after 24 hours' incubation and we have found that the most potent ε toxin is present in the filtrates of type D cultures after four or five days' incubation at 37° C.

The period of incubation is thus of considerable importance in determining the amount or potency of the different toxins. We have found that it also determines, to some extent, the type or types of toxin which may be found in a given filtrate.

Thus, when grown in test tubes of meat broth medium for a short period, the filtrates of type B cultures have been found to contain β toxin and, in some instances, ϵ toxin also. When the incubation period has been prolonged for five days ϵ has regularly been found to be the only toxin in the filtrates of these cultures.

After only a few hours' incubation in similar medium the filtrates of type D cultures have in some cases contained only α toxin or a mixture of α and ϵ. After five days' incubation ϵ has almost invariably been the only toxin detected in the filtrates.

Only a comparatively small number (11) of type C cultures have been available for study but we have found that the period of incubation does not affect the type of toxin to any marked extent as in the case of type B cultures. β toxin has been shown to be present in the culture filtrates after only a few hours' incubation and has still been detected after nine days' incubation. δ toxin has not as a rule been demonstrable in the filtrates of cultures which have been incubated for more than a few hours at 37° C.

The pH of the medium has also been found to affect toxin production to a considerable extent. During growth of type A and type D cultures, the *p*H of the medium usually falls from 7·5 to 6·5 or even 6·0. After a few days' incubation, the *p*H of type D cultures has been found to have risen towards the original level.

During the growth of type B and type C cultures, the *p*H falls rapidly to 5·0 or 4·0 and remains at this low level. We have found that when type B cultures are grown in large volumes of media with an initial *p*H of 5·0, β toxin is produced in the early stages of growth and can still be detected after several days incubation. No. ϵ toxin could be shown to have been produced at this *p*H even after a long period of incubation.

When considered in conjunction with the foregoing factors, *the volume of the medium and the proportion of meat added to the broth* have been found to influence both the potency and the type of toxin produced by the different types of cultures. We have found that highly potent β toxin can be prepared in 4 or 20 litre volumes of meat broth to which 20 per cent. of meat by volume has been added but that even after prolonged incubation little or no ϵ toxin is produced on similar media by either type B or type D cultures. When, however, tihe proportion of meat has been increased to 50 per cent. potent ϵ toxin has been produced by both type B and type D cultures. When type B cultures are grown in 4 litre or 20 litre volumes of broth containing this large proportion of meat, the β toxin deteriorates rapidly in value and apparently disappears. In some cases ϵ toxin has been found to be the only toxin in the same filtrates after a few hours' incubation.

The presence of sugar in the medium has also been found to have a marked influence on toxin production. Although the growth of each type of culture has been encouraged by the addition of glucose or lactose, we have found that certain percentages of these sugars encourage the production of β toxin but are definitely inhibitory for ϵ toxin.

More potent β toxin was found to be produced by a type B culture in a medium containing 2 per cent. glucose than in one containing 0·001 per cent. No ϵ toxin was produced by a type D culture in media containing 1 per cent. and 2 per cent. glucose when incubated for five days. Potent ϵ toxin was, however, produced in media containing no sugar or only low percentages.

The addition of trypsin to the medium in which type B cultures are grown has been found to inhibit the production of β entirely but to encourage the production of ϵ toxin in the early stages of growth. Bosworth and Glover (1934) reported that when mixtures of trypsin and ϵ toxin were incubated for a short period at 37° C. the toxicity increased considerably. There was some doubt, however, as to whether the " extra " toxin produced in this way was entirely specific. We have found that when trypsin is added to α, β or δ toxins the mixtures become atoxic after one or two hours' incubation at 37° C. Control mixtures containing these toxins and trypsin previously heated to boiling point have been found to retain their toxicity after several hours' incubation.

From the above observations it follows that an organism having the power to produce several toxins will produce only those in considerable amounts for which optimum conditions exist. We have found on some occasions that one type of toxin has been produced to the total exclusion of any other. In most cases, however, when the production of one type of toxin is encouraged, the other types are also found but may occur in such small amounts that they can only be detected by carefully controlled tests.

We would again point out the necessity for working with antitoxins the composition and value of which are known.

The Association of Toxins with Types of Cultures.

Type A culture.—Only α toxin is produced by type A cultures. These cultures can not be mistaken for any other types because of their inability to produce any other toxins. Difficulty is experienced sometimes in demonstrating toxin in some cultures, with the result that they may be classified as non-toxic strains. The production of α antitoxin after the injection of animals with filtrates may be the only evidence that α toxin or toxoid was present in such filtrates.

Type B culture.—Type B cultures are capable of producing all five types of toxins. The prevalent toxin is undoubtedly β and as a rule there is no difficulty in demonstrating its presence in cultures. If, however, all the conditions are in favour of the production of β toxin the formation of other toxins may be overlooked and the culture

erroneously classified as type C. Similarly, if the conditions favour the production of ϵ toxin and an examination of the filtrate is not made in the early stages of growth, the existence of β toxin may be missed and the culture may be wrongly classified as type D. It is very rarely that a type B organism would be classified as type A for α and β toxins are both produced in the early stages of growth. All type B cultures do not produce toxins in equal amounts nor with the same regularity. We have been able, on many occasions, to produce one toxin to the total exclusion of the others as far as the ordinary laboratory tests indicate. In spite of the fact, however, that cultures may have for many generations been encouraged to produce one type of toxin only, as a rule when now grown under other conditions they revert to their old form and produce other toxins.

We have evidence, however, that a culture of the B type can become permanently altered so that it loses its capacity for producing ϵ toxin. From a case of lamb dysentery a *Cl. welchii* culture was isolated in 1922 and after subcultures had been made, the original tube was sealed and stored. The subcultures were used for the production of toxin and each year subcultures were prepared from those in use in the previous year. On many occasions, plating was carried out and individual colonies isolated. In 1933, because of apparent differences in batches of antitoxin prepared from filtrates of different subcultures, a detailed examination was made of the latest subcultures and the parent culture. It was found that while the parent culture was a true type B, the 1933 subculture failed to produce any detectable ϵ toxin.

It has been suggested that the 1922 type B culture had become a type C culture. This does not appear to be so because :—

(1) Type C cultures produce acid when grown in glycerol and yield aldehyde and acrolein in that medium. No type B culture has behaved in these respects in our hands nor does the 1933 culture.

(2) Type C cultures have the capacity of producing large amounts of δ toxin under suitable conditions of growth. The 1933 culture produces only the amount of δ toxin associated with type B cultures.

It was undoubtedly the permanent alteration in the toxin producing ability of this culture which accounted for the production of serum containing no ϵ antitoxin and which appeared to complicate the observations of Bennetts (1932), Gill (1932) and Montgomerie and Rowlands (1934).

It has also been suggested that the original 1922 culture was really a mixture of type C and type D and that in the course of time the type D has been lost by plating. This is very unlikely for our former colleague, J. H. Mason, working in Onderstepoort, South Africa, kindly prepared for us a single cell culture of both the1922 and 1933 cultures. They behave as we have already explained. Further, if the original 1922 culture was a mixture then all type B cultures must be regarded as mixtures because all lamb dysentery strains

tested by us behave similarly as regards toxin production. Again, all type B cultures, in our hands, have failed to ferment glycerol.

Cultures of the B type do not produce large amounts of δ hæmolysin and in many cases so small is the amount that its formation can only be judged by the production of δ anti-haemolysin in animals injected with filtrates.

We have observed in connection with type B cultures that as the ε toxin is formed, there is a lessening in the amount of β toxin in the culture and a point may be reached when neither toxin is present in sufficient quantity to be detected by direct means. Under such circumstances a type B culture may be erroneously classified as a non-toxic variety of *Cl. welchii*.

Type C culture.—The characteristic feature of this group is the formation of the β type of toxin in large amounts and the occurrence of δ hæmolysin in early growths. In all, we have worked with eleven strains of type C, and on no occasion have we found any evidence of the formation of ε toxin. The presence of α toxin in type C cultures can usually only be detected with difficulty. In one of our attempts to produce toxin from a type C culture with which we had worked on many occasions, the only toxins we could detect were α and δ. It failed on that occasion entirely to produce any detectable β toxin. It is conceivable that on this examination alone, this type C culture might have been classified as a type A, especially if no tests had been carried out for the detection of δ hæmolysin.

Type D culture.—Only α and ε toxins are produced by cultures of this type. Considerable variation is found in the ability of different cultures to produce ε toxin, and it should not be considered safe to assume that ε toxin had not been produced unless the culture had been grown under the optimum conditions for at least several days. The addition of trypsin to a filtrate in which only traces of toxin are present might assist in its true indentification. Bosworth and Glover (1934).

Cultures and Toxins associated with Entero-toxaemia in Sheep.

The term entero-toxæmia is applied to a group of diseases in sheep characterised by intestinal infection with cultures of the *Cl. welchii* group. Such infections apparently occur in different parts of the world, *e.g.*, type B infections, lamb dysentery in Great Britain (Dalling, 1928), in South Africa (Mason, 1935). Type C infection in Great Britain (McEwen, 1930, Montgomerie and Rowlands, 1936), in France (Weinberg, Guillaume and Staub, 1937) and in Turkey (Weinberg, Guillaume and Forgeot, 1937). Type D infections, pulpy-kidney disease in lambs in Great Britain (Montgomerie and Rowlands, 1931), New Zealand (Gill, 1932) and Tasmania (Oxer, 1932), infectious entero-toxæmia in sheep in Australia (Bennetts, 1932). We have also identified this type of organism in material received from America and Greece from infected sheep. Apparently

the primary disease process in the majority of such infections occurs in the alimentary canal. On the other hand, cultures may in some cases be recovered from the various tissues, thus McEwen recovered *B. paludis* from the musculature of infected sheep and we ourselves isolated this organism from a sample of muscle received from McEwen; Montgomerie and Rowlands (1936) isolated the same organism from body fluids; Dalling (1928) recovered lamb dysentery strains from the liver of twelve out of many affected lambs.

It has been generally accepted that the identification of a toxin in the alimentary canal is sufficient evidence of the type of culture responsible for the infection. While this is apparently true in many cases, our observations during the past few years indicate that, especially in lamb dysentery, too much reliance must not be placed on the type of toxin found in the intestine in concluding the identity of the infecting type of *Cl. welchii*. The following table summarises our findings during the 1937 lambing season when we examined a number of carcases of lambs typically affected with lamb dysentery from a farm in Scotland. It should be pointed out that the carcases were at least 24 hours old when received.

TABLE.

Lamb.	*Types of Toxin detected in Intestinal Contents from :—*		*Types of Pure Cultures isolated from :—*		
	Duodenum.	*Ileum.*	*Stomach.*	*Duodenum.*	*Ileum.*
14	β + trace ϵ	β + ϵ	—	D	B
15	Non-toxic	β + trace ϵ	A	A	B
16	β	β	—	—	—
17	β	β + trace ϵ	—	—	—
18	β	β	D	B	B
19	β + ϵ	β	D	B	B
20	Non-toxic	β	D	B	B
21	β	β	—	—	—
23	Non-toxic	ϵ + trace β	D	B	B

No pure cultures were isolated from lambs 16, 17 and 21, but the following types of toxin were shown to be present in the first generation of cultures from the stomach and intestinal contents, after 18 hours' incubation :—

Lamb.	*Stomach.*	*Duodenum.*	*Ileum Cultures.*
16	Trace of unidentified toxin	β + ϵ	
17	ϵ	ϵ	β
21	ϵ		β

Note.—In determining the types of *Cl. welchii* in the stomach, duodenum and ileum, cultures were made in liquid media and plated. At least three single colonies were then picked from each plate and identified by their toxin production.

From the table it will be observed that: (1) different types of toxin may be found in different lambs affected with the same clinical disease condition; (2) had a diagnosis been made from the type of toxin demonstrated in the intestine, in four of the nine lambs it might have been assumed that infection had arisen from the C type; whereas culture work showed that in two of them (Nos. 18 and 20) the D type was isolated from the stomach and the B type from duodenum and ileum. Cultures were not isolated from the other two lambs (Nos. 16 and 21); (3) cultures isolated from the intestine also vary in different cases; (4) cultures also vary in individual cases at different levels of the intestine (cultures were made from the duodenum and the lower part of the small intestine; (5) cultures of toxigenic strains were isolated from the stomach; (6) generally speaking, the further forward in the alimentary canal the more is the tendency to recover types of *Cl. welchii* capable of producing ϵ toxin; (7) judging from the types of cultures isolated there is some evidence that more than one type of the *Cl. welchii* group may be present in the intestine.

From these observations it may be concluded that the identification of a toxin in the intestine of a dead lamb or sheep does not necessarily indicate the true nature of the infection. The isolation of the D type of organism from the stomach and duodenum and of the B type from the lower parts of the intestine are of considerable interest. We have no evidence to show whether the various types of *Cl. welchii* found in the intestine have a common origin and may, because of the conditions prevailing in the stomach and intestine, undergo mutation so that their capacity for producing toxins remains permanently changed. Nor is there sufficient evidence to show whether the types are entirely separate and can only survive in certain parts of the alimentary canal according to the conditions which are present.

The whole subject of the occurrence of types of *Cl. welchii* is obscure and research into the possible transformation of one type into another is indicated. In this connection, the observations of McGaughey (1933) are of importance and the fact that one strain of typical B type lost permanently its ability to produce ϵ toxin is also noteworthy.

Without much experimentation it would be difficult to ascertain the actual conditions in the alimentary canal which allow the organisms to produce infections. The mere presence of the organism in the alimentary canal is not the whole story. Most workers, using cultures of high virulence, have only succeeded in setting up infection if large doses are given, Dalling (1926), McEwen (1931), or if the normal functions of the alimentary canal are altered by the use of drugs (*e.g.*, Bennetts, 1932).

Since this article was written, Roberts has made some valuable observations concerning the production of disease in lambs by type D of the *Cl. welchii* group. (*Vet. Rec.*, **50,** 591-604.)

In the prevention of the various entero-toxæmias of sheep, vaccines and sera are largely used with gratifying results. It is inportant, however, in the absence of definite evidence of the types of toxin which the infecting organism is capable of producing that the prophylactic vaccine or serum contains adequate amounts of all the available antigens or antitoxins associated with the *Cl. welchii* group.

Summary.

(1) The types of *Cl. welchii* produce toxins by which they may be classified.

(2) Cultures vary considerably in their capacity to produce the different toxins.

(3) Certain methods encourage the production of the different types of toxin.

(4) Typing of an organism of any group cannot be said to be completed till all the methods for the production of the different types of toxin have been carried out, and until all the known tests—direct and indirect—have been applied.

(5) In cases of clinical lamb dysentery different types of *Cl. welchii* may be found at different levels of the intestine, the tendency being for the D type to occur in the more proximal part of the intestine.

(6) In the protection of sheep and lambs against an infection shown to be associated with the *Cl. welchii* group, prophylaxis against all types of toxin should be carried out unless careful tests have shown that only a certain type of organism is causing the infection.

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THE DIAGNOSIS OF STREPTOCOCCUS MASTITIS BY CULTURAL METHODS

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The results of some recent investigations have shown that the basic principle in the control of chronic mastitis due to *Str. agalactiæ* is the prevention of its spread from infected to healthy cows, particularly during milking. For this purpose it is important to detect not only clinically affected animals but also those affected with the latent form of the disease. It is essential, therefore, that a simple and reliable method of diagnosis should be available if any attempt is to be made in the future to control mastitis in dairy herds on an extensive scale.

Since indirect methods for the diagnosis of the disease do not reveal the early stages of infection, it is clear that direct cultural methods for the detection of the causal organism must be employed.

The method adopted by Minett, Stableforth and Edwards (1930) involves the cultural examination of centrifuged milk sediments by deep plating in ox-blood agar, a medium which possesses the special advantage that it permits easy recognition of infection due to strains of *Str. agalactiæ* (Group I) which give rise to hæmolytic colonies. The detection of small numbers of colonies of non-hæmolytic strains is, however, difficult in plain blood agar, particularly when contaminating organisms are present in the plate. By adding crystal violet and æsculin Edwards (1933) made this medium selective for the growth of *Str. agalactiæ* by inhibiting the growth of the common milk contaminants and by differentiating the colonial appearance of those organisms whose growth was not inhibited. In a large scale programme for the control of streptococcus mastitis, however, the present author has found this technique, although reliable, to be rather slow and laborious, the amount of glassware required and the labour involved in its cleaning and sterilisation alone being formidable. For this reason attempts have been made to devise methods which could be more simply applied and which would still give results as reliable as those obtained by the plating method.

Choice of Inoculum for Use in Cultural Work.

For the cultural examination of milk samples for the presence of *Str. agalactiæ* Minett *et al.* (1930) preferred as seed material the use of sediment, obtained by centrifuging 10 ml. amounts of milk in a modified Trommsdorf tube to the use of 0·02 ml. amounts of gravity cream obtained from the same sample after standing for two hours. By comparing these two portions obtained from 20 quarter milk samples containing beta-haemolytic streptococci, they found that larger numbers of organisms could be cultivated from the deposit than from the cream. On an average the deposits contained 30 times as many organisms as the cream. The following procedure was

therefore adopted. Where the milk sample was practically normal in appearance the centrifuged deposit was mixed with about 2 ml. normal saline of which one-half was plated directly in 10 ml. agar containing 5 per cent. of defibrinated ox-blood. The remaining half was further diluted in 9 ml. of saline of which 1 ml. was similarly plated. It will be observed that for the examination of a single sample of milk by this method the following sterile equipment is necessary: a specially constructed centrifuge tube, two plates, one pipette and one tube containing 9 ml. sterile saline.

These authors stated that an added advantage in the use of sediment for cultural work was that, by estimating its amount, confirmatory evidence was frequently obtained regarding the presence of an abnormal state of the milk as indicated by an increased cellular content. They pointed out, however, the limitations of the sediment test *per se* as a means of diagnosis, since of 326 samples from streptococcus infected quarters not more than 199 (61 per cent.) gave amounts of sediment measuring 0·01 ml. or over (this amount being arbitrarily taken as the smallest amount of sediment from 10 ml. of milk which should be classed as abnormal). Edwards (1934) examined 528 samples from infected quarters by the same test. Of these only 262 (49·6 per cent.) were judged to be abnormal. A disadvantage of the sediment test found in both these surveys was that a small proportion of milk samples from uninfected quarters also gave excessive amounts of sediment.

For these reasons the sediment test cannot be regarded as an efficient method of diagnosis and, during the routine examination of milk samples, might safely be dispensed with except for the fact that it has been shown to be a useful method of concentrating the number of streptococci in the sample.

A simpler method of preparing seed material in which the organisms were present in numbers comparable with the sediment has therefore been sought. For this purpose it was necessary to compare the streptococcus content of various portions of the same milk sample infected with typical beta-hæmolytic streptococci. Altogether 27 such samples were examined and the streptococcus counts of the following portions of the milk were compared: (1) 1 ml. of whole milk; (2) 1 ml. of gravity cream obtained by allowing the sample to stand in the ice chest for 18 hours; (3) the sediment obtained by centrifuging the sample at 2,500 r.p.m. for five minutes; and (4) 0·01 ml. of gravity cream. This quantity of cream was found to be the average amount delivered by a wire loop constructed in such a way as to give two circular loops (diam. 2·3 mm.) at its terminal end.

Technique.—From known infected quarters 20·5 ml. amounts of the foremilk were drawn into sterile marked tubes and 10·5 ml. from each sample poured into modified Trommsdorf centrifuge tubes. After storage for 18 hours in the ice chest the milks were removed and after thorough shaking 0·5 ml. was taken from each centrifuge tube for the estimation of the count in whole milk while the remainder was centrifuged at 2,500 r.p.m. for five minutes and the amount of sediment determined. The sediment was well mixed with 0·5 ml. saline and three serial dilutions made in 9·5 ml. amounts of saline; from each saline dilution 1 ml. was plated in ox-blood agar. From the other 10 ml. portion of the milk one loopful (approximately 0·01 ml.) of the accumulated gravity cream was removed into 2 ml. of saline and

1 ml. of the resulting emulsion plated, while the other 1 ml. was diluted into 9 ml. of saline and 1 ml. of this added to a second plate. Lastly, 0·5 ml. of the gravity cream was removed and a separate pipette used for making three serial dilutions in saline, which were subsequently plated.

TABLE I.
NUMBERS OF STREPTOCOCCI IN VARIOUS PORTIONS OF THE SAME MILK SAMPLE.

	Sample No.	*Milk per ml.*	*Cream per ml.*	*Cream Loop (approximately ·01 ml.)*	*Sediment (from 10 ml. Milk).*	*Amount of Sediment.*
C	4	42,400	107,200 (2·5)	3,380	8,500 (2·2)	Trace
	8	16,200	22,600 (1·4)	2,060	2,080 (1·0)	,,
	9	3,840	18,000 (4·7)	960	260 (0·25)	,,
D	19	23,600	13,200 (0·6)	454	690 (1·52)	,,
	20	196,000	372,000 (1·9)	11,580	54,000 (4·6)	,,
	25	8,720	32,400 (2·7)	584	2,130 (3·64)	,,
C	2	3,480	21,400 (6·1)	306	480 (1·56)	Slight
	6	132,400	179,000 (1·4)	15,000	36,400 (2·43)	,,
D	18	32,800	118,000 (3·5)	1,900	6,700 (3·52)	,,
	22	62,800	174,000 (2·8)	6,080	21,600 (3·55)	,,
	23	24,600	63,200 (2·6)	4,160	24,600 (5·91)	,,
	24	20,600	30,800 (1·5)	2,080	4,100 (1·97)	,,
D_2	27	43,400	93,000 (2·1)	5,260	16,100 (3·06)	,,
					Average (2·7)	
C	1	2,540	11,200 (4·4)	144	1,000 (6·9)	0·01 ml.
	3	6,240	21,800 (3·5)	328	2,240 (6·8)	,,
D	15	4,960	12,200 (2·5)	254	1,480 (5·8)	,,
	17	3,440	21,200 (6·1)	244	1,100 (4·5)	,,
D_2	26	3,120	15,200 (4·9)	252	2,440 (9·68)	,,
					Average (6·7)	
D	11	11,200	42,000 (3·7)	452	4,900 (10·8)	0·015 ml.
D_2	28	5,680	49,600 (8·7)	420	10,900 (25·9)	,,
D	13	6,040	9,200 (1·5)	218	2,470 (11·3)	0·02 ml.
C	10	4,880	9,800 (2·0)	472	2,840 (6·02)	0·03 ml.
D	12	61,200	132,000 (2·2)	2,340	41,600 (17·76)	,,
C	7	21,600	104,400 (4·7)	1,560	17,000 (10·9)	,,
D	14	3,760	13,600 (3·6)	252	2,510 (9·95)	,,
	16	2,920	8,400 (2·9)	68	1,240 (18·2)	,,
	21	70,600	144,000 (2·0)	2,450	89,000 (36·1)	,,
			Average (3·2)		*Average* (16·3)	

In Table I the streptococcus counts of the various portions of the 27 infected samples are shown. The figures in brackets in column 3 represent the ratio of the counts in 1 ml. of the gravity cream to those in the same volume of whole milk, while the figures in column 5 show the similar ratio between sediment and 0·01 ml. of gravity cream. It will be seen that gravity cream gave consistently higher counts than whole milk and that the average ratio was 3·2 : 1. These results support those of Pullinger (1935) who also found that cream was richer in organisms than whole milk. In the present work no sample was found in which organisms were absent from the whole milk when they could be cultivated from the gravity cream—a fact which was observed by Pullinger.

Comparing the counts observed in the sediment and in 0·01 ml.

of gravity cream it will be seen that higher counts were invariable found in the former. The average ratio of the counts in these two portions was, however, 2·7 : 1 when the sediment of the sample was less than 0·01 ml., whereas in samples having a sediment over 0·01 ml. the average ratio was 16·3 : 1. Nevertheless, even in the latter series of samples the difference in numbers of organisms in the sediment and in gravity cream was not so great as those recorded by Minett *et al.* (1920). Since, as has been pointed out earlier, about one-half of the quarters affected with latent mastitis yield sediments below 0·01 ml. from 10 ml. of milk, it would seem justifiable, in the routine examination of milk samples for the detection of *Str. agalactiæ*, to expect the counts, using 0·01 ml. of gravity cream as seed material, to be of the same order as those obtained from sediment. Furthermore, it is obvious that the use of a small volume of gravity cream which can be conveniently delivered by means of a wire loop has advantages over other methods of inoculation since a marked economy in time and material is effected in seeding out the samples.

For this reason cultural examination of milk samples for the presence of mastitis streptococci has been carried out in the following manner. A standard loop* has been used for the inoculation of 0·01 ml. gravity cream from each sample into one plate containing a small quantity of sterile saline. The cream was emulsified, approximately 10 ml. of the selective blood agar medium added, and the plate well shaken to ensure even mixing. This technique has been used as a standard method throughout the following work and has formed a basis for assessing the comparative value of the tests to be described later.

The Diagnosis of Streptococcus Mastitis by the Growth in Incubated Milk Samples.

In the present investigation 829 samples from individual quarters of the cows in six herds were drawn with reasonable precautions to avoid external contamination into small tubes measuring 11 cm. by 1·3 c.m. The udder of each animal was previously washed and dried as is customary before milking. In order to prevent the development of growth due to staphylococci each sample tube contained crystal violet together with a sufficient quantity of brom-cresol-purple indicator to give a satisfactory colour when the milk was added. The amount of milk drawn into the tubes under normal conditions of sampling was found to vary between 5 ml. and 8 ml., with an average of 7·5 ml. Accordingly the amount of crystal violet added to the tubes before sampling was calculated to give a final concentration of 1/750,000. This concentration was found to be optimal for the inhibition of moderate numbers of staphylococci. In practice a

* The variation in the volume of cream and in the number of streptococci delivered by three successive loops from three separate milk samples was determined. This variation was found to be so small that for practical purposes the volume of the loop could be considered constant.

solution of equal parts of crystal violet (1/20,000) and brom-cresol-purple (0·1 per cent.) was made up. These solutions were sterilised separately, mixed after cooling and then 0·4 ml. was added to each sterile tube. The writer took all the samples personally by drawing about one stream of fore-milk into a tube held as horizontally as possible, the rubber stopper being immediately replaced by an assistant. The samples were kept in a cool place overnight and on the next morning, after one loopful (0·01 ml.) of the gravity cream had been removed for plating in blood agar, they were placed in the incubator at 37° C. After incubation for 48 hours the milk tubes and the plates were read independently. Bacterial growth in the milk was denoted by a yellowish-white coloration, which had usually developed throughout the whole medium after this period of incubation. This change in colour was not necessarily accompanied by clotting of the milk. Streptococci of the mastitis Groups I, II and III were looked for in the plates and suspicious colonies were verified in test media. In Table II the growths present in the milk sample are compared with the bacteriological examination of the same samples in blood agar.

Table II.

	Samples giving Negative Results on Cultural (Plate) Tests.	*Samples giving Positive Results on Cultural (Plate) Tests.*
Number of samples giving negative results in milk tubes	632 (83 per cent.)	0
Number of samples giving positive results in milk tubes	131 (17 per cent.)	66 (100 per cent.)
Total	763	66

It will be observed that with 131 (17 per cent.) of the 763 samples in which no mastitis streptococci were found in the plates, positive readings were recorded in the milk, while all the samples in which the organisms were found in the plates showed growth in the milk. When the figure 131 is analysed, however, it is found that 56 occurred in one herd while the remaining 75 were distributed fairly evenly over the remaining five herds. The conditions of sampling in the first herd precluded the chances of sterile conditions being achieved since the hygienic conditions in the byre were bad. Excluding this herd the total number of quarter samples in which the plates showed no streptococci was 630, the milk tubes being positive in 75 samples, or 12 per cent.

It may be claimed, therefore, that the direct milk incubation test gives roughly 10 per cent. erroneous results when the conditions for sampling are good, but when these conditions are unfavourable the results are seriously invalidated by the growth of extraneous organisms.

It might be argued that, since the results were based on a comparison with the growth of organisms from only one loopful of gravity cream seeded in blood agar, the positive findings in the milk were in fact due to the growth of mastitis organisms undetected in the plates. This is hardly likely to have been the case, since the milk was derived from cows which belonged to herds included in a mastitis control scheme, and the history of the presence of disease could, therefore, be checked from the results of several examinations.

The above test will probably give fairly reliable results when it is possible for a trained worker to visit suitable herds for the purpose of taking milk samples, but if such sampling is left to the farmer himself there appears to be little chance of avoiding an unduly heavy contamination in the samples. A further disadvantage of this method is that when additional information regarding the type of streptococcus responsible for the growth is required, subsequent plating, isolation and verification of colonies in various test media must be carried out.

White the work on this subject was in progress a report of a similar investigation was published by Hotis (1936). This author recorded that successful results were obtained from direct incubation of milk samples, although no previous addition of crystal violet was made in his investigation. By taking samples with aseptic precautions he obtained 95 per cent. agreement between the results of direct milk incubation and cultivation in blood agar plates. Bryan and Devereux (1937) failed, however, to confirm such a close agreement and many doubtful readings were obtained. While the percentage agreement of the present series was 83, it appeared possible that, by using an even more selective medium, closer agreement might be reached. With this end in view the following experiments were undertaken.

The Diagnosis of Streptococcus Mastitis with a Liquid Selective Medium.

The use of a liquid medium such as glucose broth for the detection of mastitis streptococci has several advantages over deep cultivation in blood agar for large-scale work. The preparation of the liquid medium is simple and it may be stored in tubes ready for use. Moreover, the presence of small numbers of streptococci, especially of those which give rise to non-haemolytic colonies, is more easily detected. Details of such a medium were described by the writer (1930, 1933).

While this glucose broth medium was inhibitory to the growth of many Gram-positive organisms and was found to be a useful aid in the detection of small numbers of mastitis streptococci, it did not prevent the growth of Gram-negative organisms, such as *Bact. coli*, which are frequently present in milk samples. Hartmann (1936) investigated the value of a number of substances for the selective cultivation of mastitis streptococci from contaminated material. Of

those which were inhibitory to *Bact. coli* in concentrations which permitted the growth of *Str. agalactiæ*, sodium azide was found to be the most suitable. In agar containing 10 per cent. defibrinated blood and 1 per cent. glucose a concentration of 1/5,000 inhibited strains of coliform and proteus organisms, while the growth of several strains of mastitis streptococci was unhindered.

It was thought possible that the addition of sodium azide in suitable concentration to the glucose broth medium would form a highly selective medium for the growth of mastitis streptococci. Accordingly, experiments were carried out to determine the effect of various concentrations ranging from 1/5,000 to 1/20,000 of sodium azide on (1) the growth of small numbers of streptococci, (2) the growth of various strains of *Bact. coli*. The optimum concentration appeared to be 1/10,000 but it was found on investigation that the *p*H of the medium markedly affected not only the streptococcus growth but also the inhibitory action of the azide on *Bact. coli.* strains.

Technique.—In this experiment the basic medium consisted of meat extract broth to which glucose 0·5 per cent. and crystal violet (1 part in 1 million) was added. The *p*H of one batch of medium was adjusted to 7·0, while the other was adjusted to 7·6. To one-half of the medium in each batch sufficient sodium azide to give a concentration of 1/10,000 was added while the other half was kept as a control medium. The four lots of media were each filled into tubes 11 cm. by 1·3 cm. with approximately 6 ml. of medium, and sterilised by autoclaving at one atmosphere for ten minutes. The final *p*H values of the two batches were then 6·8 and 7·4 respectively. Suitable dilutions of three strains of beta-haemolytic streptococci were made in 9·5 ml. amounts of saline and from the last dilution a small measured amount was placed in a plate to which blood agar was added, while the same quantity was added to the four series of liquid media. Similarly, dilutions

TABLE III.

THE INFLUENCE OF *p*H OF SELECTIVE MEDIUM ON THE GROWTH OF (1) SMALL NUMBERS OF MASTITIS STREPTOCOCCI, AND (2) LARGE NUMBERS OF *Bact. coli.*

No. of Organisms in Blood Agar Plates.			*Medium Alone.*	*Medium + Azide* 1/10,000.		*Medium Alone.*	*Medium + Azide* 1/10,000.	
			24 *Hours.*	48 *Hours.*	24 *Hours.*	48 *Hours.*	24 *Hours.*	48 *Hours.*	24 *Hours.*	48 *Hours.*
B. strep.	1	135	+++	+++	+	+++	O	+++	O	+
	2	31	++	+++	+	+++	O	++	O	++
	3	39	++	+++	+	++	O	++	O	++
Bact. coli	1	Inl.	+++	+++	O	O	+++	+++	±	+
	2	„	+++	+++	O	O	+++	+++	±	+
	3	„	+++	+++	O	O	+++	+++	±	+
	4	„	+	++	O	O	±	++	O	O
	5	„	+++	+++	O	O	±	++	O	+
	6	„	+++	+++	O	O	+++	+++	O	±
	7	„	+++	+++	O	O	+++	+++	O	O
				*p*H 6·8			*p*H 7·4			

of seven *Bact. coli* strains isolated from milk and from faeces were made and small volumes employed as for the streptococcus strains. The growths developing in the liquid media were read after 24 hours incubation and again after a further 24 hours and the degree of growth was indicated as follows : O, no growth ; ±, trace ; +, slight ; ++, moderate ; +++, abundant.

It can be seen from Table III that in the medium with the *p*H. of 6·8, the development of streptococcus growth was quicker and more profuse than in the medium with a *p*H of 7·4, while the inhibitory action of azide on the coliform strains was considerably augmented. Further, this concentration of azide was bactericidal to numbers of coliform organisms which would be in excess of those ordinarily encountered in milk.

It was decided, therefore, to use a medium having the following composition for the selective cultivation of mastitis streptococci from milk samples.

Meat extract (Lemco) broth (*p*H 7·0)	1,000 ml.
glucose	5 gr.
Crystal violet (purified) 0·1 per cent.	1 ml.
sodium azide	0·1 gr.

Approximately 6 ml. of medium were filled into tubes measuring 11 cm. by 1·3 cm. and autoclaved at a pressure of one atmosphere for 10 minutes. The final *p*H of the medium was 6·8.

The Action of Sodium azide on Str. agalactiæ and Bact. coli in Milk during Storage.

It might be thought that a method of diagnosis could be employed involving the direct incubation of samples of milk containing sodium azide instead of crystal violet, but numerous observations on this point showed that a large number of indefinite readings were liable

Table IV.

The Effect of Sodium Azide on Streptococcus and *Bact. coli* Counts during Storage in Milk at 24° C. for 24 Hours.

Sample.		*Before Storage.*	*After Storage.*		
			Alone.	1/10,000 *Azide and b.c.p.*	1/20,000 *Azide and b.c.p.*
Milks infected with *B. strep.* ...	1	980	Uncountable	4,400	56,200
	2	910	„	14,700	244,000
	3	620	4,600	2,650	4,300
	4	85	Uncountable	2,400	40,300
Milks sown with *Bact. coli* strains	1	52,900	Uncountable	19,600	52,000
	2	256,000	„	4,800	18,400
	3	19,300	„	1,140	1,440
	4	14,400	„	750	1,520
	5	9,900	„	720	4,880

to be obtained. If, however, it could be shown that the addition of this substance to milk samples would prevent the development of coliform organisms while leaving the numbers of streptococci unchanged, it would obviously be advantageous to include it in samples which were being despatched to the laboratory, especially during the summer months.

The results of an experiment designed to test this point are recorded in Table IV.

Four milk samples were taken from the quarters of cows naturally infected with beta-haemolytic streptococci and each sample was divided into three equal amounts of 7·5 ml. To one series of samples sodium azide was added to a concentration of 1/20,000, while the third series was kept as a control. The first and second series also contained brom-cresol-purple indicator (0·13 ml., 0·1 per cent. solution in each tube). Three series containing similar concentrations of azide were prepared from milk which had been heated at 60° C. for 30 minutes. Into one tube of each series a small volume of a suitable saline dilution of a *Bact. coli* strain isolated from faeces was inoculated and the tube was thoroughly shaken. Altogether five such strains were used.

The bacterial count of the control series before storage was determined by plating dilutions of the milk in blood agar, and after this the three series of tubes were placed in an incubator at 24° C. where they were maintained for 24 hours. The counts of each series were again estimated.

In three of the four samples the two concentrations of azide used prevented the numbers of streptococci from developing to the same extent as in the milk alone during storage, though the counts after storage were in all instances very much greater than the initial counts. On the other hand a marked inhibitory action on the growth of the coliform strains was produced in both series, the counts after storage being markedly less than in the control samples stored for the same period and considerably reduced even from those in the original samples.

This experiment showed, therefore, that considerable advantages attended the addition of sodium azide to milk samples for the prevention of the development of coliform organisms during transit to the laboratory at summer temperatures. A concentration of 1/20,000 was chosen since at this strength the streptococcus count was unlikely to be affected while the growth of *Bact. coli* would be inhibited. For this reason the following routine procedure has been adopted in this laboratory in preparing tubes for sampling the individual quarters of cows for the diagnosis of streptococcus mastitis. To sterile tubes measuring 11 cm. by 1·3 cm., 0·5 ml. of the following mixture is added: 37 parts sodium azide (0·1 per cent.) and 13 parts brom-cresol-purple (0·1 per cent.) Assuming that the average amount of milk drawn into a sample tube is 7·5 ml. the concentration of sodium azide will be 1/20,000 and the brom-cresol-purple will also be in sufficient strength to impart a satisfactory colour so that the reaction of the milk can be seen at a glance.

Comparison of Blood Agar Plating and the Liquid Medium Culture of Milk Samples in the Detection of Mastitis Streptococci.

In the first place 1,014 milk samples from the individual quarters of cows belonging to five herds in which a programme for the control of streptococcus mastitis was in progress were examined by the following methods:—

Milk samples were taken by the author into tubes prepared according to the preceding section and after storage in the laboratory overnight were cultured by the two methods. Into the liquid selective medium 0·01 ml. of the gravity cream was inoculated with a wire loop in such a way that the cream remained as far as possible on the surface of the medium. Simultaneously, a similar amount was sown into a plate containing about 1 ml. of saline in which the cream was emulsified. Approximately 10 ml. of the blood agar medium described by the writer (1933) was run into the plates and the contents well shaken. Both the liquid medium and the plates were incubated at 37° C. for 48 hours and were then read independently.

In the liquid medium the cream gave rise to some turbidity after 24 hours' incubation, but after the further period it appeared at the surface where its presence did not interfere with the reading of streptococcus growths which usually developed as flocculent deposits after 48 hours' incubation. In the blood agar plates examination was made for the presence of the mastitis streptococci belonging to Groups I, II or III. Colonies showing the typical beta-haemolytic zones of *Str. agalactiae* (Group I) were not examined further but representative non-haemolytic colonies were picked into broth and identified by their reaction in methylene blue milk (1/20,000) and their ability to ferment mannite and salicin. Similarly, the growths which had developed in the liquid selective medium were sown into the same test media. These growths were not verified by microscopic examination.

A further series of 366 mixed quarter milk samples were taken by the owners of nine different herds into prepared tubes and were examined by the two methods. With most of these samples the interval between the times of taking and testing in the laboratory was considerably longer than in the first series. Generally a period of 18 hours or so elapsed during transit to the laboratory, and after arrival the milks were allowed to stand for a similar period before testing in order to permit the accumulation of gravity cream.

The results of the two series of examinations are shown in Table V. It will be seen that the agreement between the readings in the blood agar plates and in the broth medium over the whole series is high, being 96·1 per cent. in the quarter samples and 93·4 per cent. in the mixed samples. Referring to the quarter samples classed as positive to the two tests there was agreement in the streptococcus group identified by the two tests in 90 out of 95 samples. Similar results were obtained with 93 out of 102 mixed samples.

In 29 quarter samples mastitis streptococci were found in the broth medium when none were recognised in the plates and 13 mixed samples gave similar results. In these instances it is possible that

Table V.

Comparison of Results obtained in Blood Agar Plates and in the Liquid Selective Medium.

	Plates and Broth both Positive.			*Plates Negative.*				
				Broth Negative.	*Broth Positive.*			
	Group 1.	*Group* 2.	*Group* 3.		*Group* 1.	*Group* 2.	*Group* 3.	*Group* ?.
Quarter samples 1014 ...	74 (1)	11	10 (4)	885	10	0	19	*Unidentified.* 5
Mixed samples 366 ...	89 (6)	5 (1)	8 (2)	249	9	2	2	2

The figures in brackets indicate the number of anomalous results, *i.e.*, samples where the streptococcus groups identified in the broth and corresponding plate were not the same. These samples have, therefore, been omitted from the totals in the calculations of agreement which are as follows :—

$$\text{Agreement in quarter samples} = \frac{975}{1014} = 96{\cdot}1 \text{ per cent.}$$

$$\text{Agreement in mixed samples} = \frac{342}{366} = 93{\cdot}4 \text{ per cent.}$$

very small numbers of non-hæmolytic streptococci could not be recognised in the corresponding plates. Only five growths in the broth medium sown from quarter samples could not be identified as belonging to any of the mastitis groups, while two growths from the mixed samples could not be identified. In no case, however, did the broth fail to show the presence of streptococci which could be detected in the corresponding plates.

Discussion.

It is clear from the foregoing results that the liquid selective medium described and the technique evolved for its use deserve a place in the routine diagnosis of streptococcus mastitis. It remains to be stated how far the various methods should be applied as an essential part of a programme for the control of the disease in dairy herds.

The special advantage of deep cultivation in blood agar is that infections due to *Str. agalactiæ* (Group 1) strains which are beta-hæmolytic are easily recognised, and in such instances no subsequent identification of the colonies is necessary. Infection due to strains of this kind is a more common cause of chronic mastitis in herds than that due to non-hæmolytic strains. Thus Minett *et al.* (1929) found that of 82 clinical cases of bovine mastitis due to streptococci, 52 (63·4 per cent.) of the strains were hæmolytic and 30 non-hæmolytic.

Edwards (1934) found that of 619 strains of mastitis Group 1 streptococci isolated from quarter milk samples of cows in 18 herds, 475 (76 per cent.) were beta-hæmolytic and 44 (24 per cent.) non-haemolytic. In the present investigation 38 out of 74 (51 per cent.) quarter samples showed beta-hæmolytic colonies, while 54 out of 89 (65 per cent.) mixed samples were similarly infected.

Therefore, in view of the predominance of infection due to hæmolytic strains and the suitability of the blood agar plate method in revealing infection due to these organisms, it is considered that at the first examination of a herd, cultural examination of milk samples should be made by deep plating in a blood agar medium. For the examination of the clean section of the herd at subsequent tests economy of labour may be effected by using the liquid medium alone.

The following laboratory procedure may, therefore, be recommended in the control of streptococcus mastitis:—

(1) At the first test 20 ml. composite milk samples from the udders of each cow are drawn at evening milking into tubes containing 1 ml. of a mixture of equal parts of sodium azide solution 1/500 and brom-cresol-purple solution 0·1 per cent. The final concentration of sodium azide in the sample will then be 1/20,000 and sufficient brom-cresol-purple will be added to give a satisfactory colour to judge the reaction of the milk. The samples may be kept at room temperature until the next day when the reactions of the milk are read and 0·01 ml. of the gravity cream inoculated by means of a wire loop into a plate and the same amount into the liquid selective medium. Into the plate a little saline should have been added previously in order to emulsify the cream. About 10 ml. of crystal violet-æsculin-blood agar are added and the plate well shaken to ensure even mixing. After 48 hours incubation the plates and broth are read. Plates from samples containing beta-hæmolytic streptococci require no further examination. The positive readings in the broth will be confirmatory and, in addition, will direct attention to plates containing the colonies of non-hæmolytic streptococci.

(2) At the next test, which should take place two months after the first, a more thorough examination of the clean section of the herd should be carried out by examining quarter milk samples. These should be drawn into small tubes measuring 11 cm. by 3 cm., azide and brom-cresol-purple in the correct concentrations having been added to the tubes previous to sampling.

On this occasion 0·01 ml. of gravity cream should be sown carefully into the liquid medium only and note made of growths which have developed after 48 hours incubation. Growths of *Str. agalactiæ* are recognised by large flocculent deposits with complete clearing of the supernatant liquid. Subsequent identification may be confirmed by the action on methylene blue milk (1/20,000) and the ability to ferment mannite and salicin. If necessary, the culture may be plated

in blood agar in order to determine whether colonies are hæmolytic or not.

(3) Subsequent tests on the clean section of the herd may be carried out on composite milk samples sown into the liquid medium, the nature of the growths being verified as before.

Recently a routine procedure for the laboratory detection of bovine mastitis has been outlined by Plastridge *et al.* (1938).

> The method depends essentially on the recognition of surface colonies of *Str. agalactiae* on the medium described by Edwards (1933) where, according to the authors, they can be differentiated from other types of streptococci occurring in milk. This medium is inoculated by streaking 4 mm. loopfuls of milk samples which, after incubation for 16 to 20 hours, have shown streptococci on microscopic examination. In addition, samples which are free from streptococci, but which show the presence of 500,000 or more leucocytes per ml. are similarly cultured both on the selective medium and on plain ox-blood agar. The use of the plain medium is intended to reveal the presence of infection due to *C. pyogenes* or haemolytic staphylococci. The milk samples are classified as negative, suspicious or positive, according to the results of presumptive tests (leucocyte count, reaction to bromthymol blue, physical appearance), microscopic examination of incubated samples and the nature of the colonies found on the blood agar medium. Samples which yield grey-blue streptococcus-like colonies without browning of the medium are confidently recognised to be infected with *Str. agalactiae.*

The procedure suggested by Plastridge *et al.* certainly merits comparison with that described in this paper. Without having applied the two methods side by side for the examination of a large number of milk samples it is not meantime possible to compare the time and materials required and the accuracy of the results achieved. One comment may, however, be made regarding the necessity of examining milk samples on plain blood agar for the presence of organisms other than streptococci when an abnormal cell content is detected. As regards *C. pyogenes*, experience obtained at this Institute indicates that when this organism is present the milk is grossly altered and there is an accompanying clinical history from the effected animal. Such samples have never been obtained from lactating cows during the present programme of mastitis control. Again, experience has shown that infection due to hæmolytic staphylococci is seldom met with as the cause of a mild inflammatory process simulating that produced by *Str. agalactiæ*. On the other hand a few cases of staphylococcus infection accompanied by acute symptoms with marked changes in the milk have been encountered. These facts indicate, therefore, that organisms other than streptococci produce marked physical changes in the milk and that only in such conditions is it necessary to resort to cultivation in plain blood agar.

Summary.

(1) In this paper methods have been devised to facilitate the bacteriological diagnosis of chronic streptococcus mastitis in quarter and composite milk samples.

(2) The streptococcus contents of the whole milk, sediment and gravity cream have been compared and it is suggested that a small quantity of gravity cream (0·01 ml.) is satisfactory and may conveniently be used as seed material.

(3) When compared with cultural examination in blood agar plates, direct incubation of samples for the detection of *Str. agalactiæ* did not give reliable results since non-specific growths were frequently encountered.

(4) For the selective cultivation of *Str. agalactiæ,* however, a glucose broth medium containing crystal violet (1/1,000,000) and sodium azide (1/10,000) was successfully employed and proved more convenient for routine use than the plating method.

(5) A procedure based on the application of this method and designed to facilitate the working of a scheme for the control of chronic mastitis is outlined.

It is a pleasure to acknowledge the interest and helpful criticisms of Dr. N. C. Wright and Dr. F. C. Minett throughout the course of this work. Thanks are also due to the Agricultural Research Council who provided special grants to cover the cost of the investigation.

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RINDERPEST IN AFRICAN GAME

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Cattle plague probably causes a greater mortality in domestic stock than any other disease in Tropical Africa. Unfortunately rinderpest is not confined to cattle, but is known to occur in many species of wild ungulate game which inhabit vast areas of this continent.

As will be readily imagined, this susceptibility of the wild fauna proves a serious complicating factor in the control of rinderpest in the many countries where the disease exists; and in the earliest recorded epizootic of 1889, which invaded Africa at Eritrea, there is no doubt that the presence of susceptible game was in no small measure responsible for the sweep south through East and Equatorial Africa to Cape Colony.

The earlier writers such as Lugard and Sharpe mention buffalo, eland, warthog and wild pig as being the chief victims, and at the present time these animals are generally regarded as being universally of high susceptibility, although this short list by no means exhausts the class of wild animals which suffer from rinderpest. Specific susceptibility of game seems to vary considerably at different times and in different localities, whilst the natural habits of certain species affect to a greater or lesser degree their rôle in so far as dissemination in domestic stock is concerned.

Poulton (1927) gives the following list of game in Uganda and their susceptibility to rinderpest:—

Elephant—Insusceptible.
Rhinoceros—ditto.
Hippo—ditto.
Buffalo—Highly susceptible.
Giraffe—ditto.
Eland—ditto.
Roan—Insusceptible or only slightly so.
Waterbuck—ditto ditto.
Hartebeest—ditto ditto.
Topi—ditto ditto.
Kob—Varying from high to slight susceptibility.
Bushbuck and Sitatunga—ditto ditto.
Reedbuck—Slight.
Mpala—Variable.
Oribi—Variable, losses usually slight if any.
Duikers—ditto ditto.
Giant Hog—Susceptible—observations nil.
Pig—Highly susceptible.
Wart-hog—ditto.

The Blue Wildebeeste (*Connochætes taurinus*) has been proved to be susceptible in Tanganyika, and observations are recorded by

Cornell (1933) in connection with *post-mortem* details and sub-inoculation experiments.

The Koodoo are known to be highly susceptible and there are numerous references to heavy mortality in these beautiful antelope during the great epizootic of the late nineties of last century. In a letter to the *Field*, Selous (1908) refers to deaths in koodoos in the Eastern Transvaal about 1896, whilst Fox and Varian (1911) in an article on game in Angola mention the deaths amongst koodoo in East and Central Africa during the same epizootic, which incidentally did not reach Angola.

Observations in Uganda.

Buffalo.

From the point of view of control there is no doubt that the buffalo is the most important wild disseminator of rinderpest in Uganda. They are almost ubiquitous in their distribution and frequently graze over the cattle pastures during the night, while their propensity for scattering and travelling long distances after infection has been established in a herd is well known. An interesting and well-known fact is that buffalo, normally of a retiring disposition, will become dangerously aggressive when ill with rinderpest, and several fatalities have occurred following unprovoked attacks on unfortunate individuals who visit a waterhole frequented by a rinderpest-infected buffalo.

It has been recorded on several occasions in Uganda by reliable observers, and I myself have noticed on more than one occasion, that cattle known to have been infected through buffalo often show a markedly lower mortality rate than that generally experienced by other herds in the same epizootic area.

On one occasion the death rate dropped from 80 per cent. down to 47 per cent., while Allan (1930) in the Toro district reports a decline from approximately 90 per cent. to 40 per cent.

There is also evidence to show that the mortality again rises to normal as the disease spreads through cattle. This phenomenon would appear to be due to an alteration in the virulence of the rinderpest virus, as other factors such as susceptibility, type of cattle, weather conditions, etc., remain constant. The difficulty of scientific proof in such cases is obvious, and one must rely on field observations. There has, however, been evidence adduced in connection with other species of game that either the virulence remains unchanged or appears to be exacerbated, but again scientific proof is hedged by numerous practical difficulties. These cases will be discussed later.

Genus Kobus.

Waterbuck (Kobus ellipsiprymus) (Kobus defassa).

It is generally assumed that this species of *Kobus* is rarely infected with rinderpest and no authenticated case of a natural infection has been recorded. Mettam (1936) inoculated virus into a waterbuck

at this Laboratory and the animal passed through a severe attack of the disease but did not succumb. These antelope are rather local in their distribution and would not in the ordinary course of events prove a menace to cattle.

Uganda Cob (Kobus thomasi).

Poulton (1918) reported large numbers of *Kobus thomasi* infected on the Mayanja flats in Uganda; the mortality was high and large herds were decimated. In 1926, on the Nile flats in the West Nile District, rinderpest killed off buffalo in large numbers but the cob, of which there were a fair number, did not appear to suffer. This variation in the morbidity rate is difficult to explain and whether it is an alteration in the virus or a change in susceptibity of the host is at the moment impossible to determine.

The only recorded experiment on this latter species was carried out at this Laboratory (Mettam, 1936) and the animal, a full-grown male, passed through a severe attack of the disease after inoculation with a known bovine virus. It, however, made a fairly rapid recovery and it was established, as far as single inoculation can be evaluated, that both the cob and waterbuck appear more resistant to rinderpest than the local cattle, which die regularly in 8—12 days following the injection of virus.

Genetra Tragephalus and Limnotragus.

Sitatunga (spekei) and Bushbuck (T. sylvaticus).

Both these species are well known as being generally highly susceptible to rinderpest. Their habitat is local and they do not constitute an important factor in the spread of the disease.

A natural outbreak in the game animals at this laboratory provided an opportunity of closer study of infection in this genus and the following observations. A full-grown male bushbuck was reported sick on 10.6.1937. It was given 100 c.c. of anti-rinderpest serum but symptoms of rinderpest developed and the animal died on 16.6.1937. In 1934 virus was recovered from a case of rinderpest in a bushbuck at the laboratory and it proved fully virulent to cattle. Three sitatunga also became infected : the course of the illness was rapid, and except for diarrhoea and staring coat no other symptoms were observed.

(*a*) *Adult male sitatunga.*—First noticed to be ill on 20.5.1937. Died 22.5.1937. *Post-mortem* examination showed a very slight stomatitis and pharyngitis but the abomasum and small intestines were acutely inflamed and hæmorrhagic.

(*b*) *Young sitatunga.*—This was a half-grown buck and although under close observation was only noticed to be ailing one day before it died.

(*c*) *Adult female sitatunga.*—First showed signs of illness on 26.5.1937, with a rough coat and diarrhoea. It died on the night of 28—29.5.1937.

Post-mortem examinations on these latter cases revealed inflammatory changes in the mucous membrane of the hard and soft palates and oesophagus. The abomasum was acutely inflamed and the mucous membrane red, swollen and œdematous, and numerous small circumscribed ulcers, round and sickle shaped, occurred in the cardiac portion of this organ. There was acute or sub-acute enteritis throughout the intestinal tract. The diagnosis of rinderpest in the sitatunga was confirmed by sub-inoculations of blood, and spleen pulp into known susceptible and immune bovines. Of these, calf No. 2156 gave a marked temperature reaction extending over 15 days with mild buccal lesions and recovered. It is interesting to compare this animal with that inoculated with Reedbuck material described later in this paper. The other susceptible animal, No. 2911, showed a temperature reaction only and later when tested with virus proved immune.

Genera Redunca (*Cervicapra*) *and Rhaphiceros.*

Reedbuck and Oribi.—Antelope belonging to both these genera have always been considered as immune to rinderpest or at any rate highly resistant. There is no authentic record of infection under natural conditions and it is a common sight in certain parts of the country to see these delightful animals living and apparently healthy in rinderpest quarantines—areas of concentrated infection. The following experience at the laboratory is therefore of interest:—

(*a*) *Adult Reedbuck* found dead in game paddock 13.5.37. Reported as healthy the day before. *Post-mortem* examination showed severe gastritis but no mouth lesions. Examination for anthrax was negative and the cause of death was recorded as acute gastritis of unknown ætiology. As later events proved this animal undoubtedly died of rinderpest.

(*b*) *Young Reedbuck* found dead 26.5.37. *Post-mortem* examination showed a diphtheritic deposit, and commencing ulceration in the mouth. The mucous membrane of the abomasum was acutely inflamed, haemorrhagic and œdematous. Susceptible calf No. 2830 received 15 c.c. of spleen emulsion on 26.5.37. Its temperature was 107° F. on the fourth day and it died of peracute rinderpest on the sixth day after inoculation. Spleen emulsion from this animal was inoculated into a further calf, No. 2849, which died of acute rinderpest on the tenth day. It is interesting to compare these very severe reactions, especially calf 2830, with the inoculation from the sitatunga recorded above which showed quite a slight manifestation of the disease. These calves were approximately of the same age and breeding and were kept under identically the same conditions.

Oribi.—This was an adult male and was sick for one day only, showing symptoms of diarrhoea. *Post-mortem* examination revealed gastro-enteritis and numerous small sub-endocardial petechial hæmorrhages. There were no lesions on the mouth. Sub-inoculation into a susceptible bovine proved the presence of rinderpest.

Eland (*Taurotragus sp.*).

These large antelope were one of the earliest species to be recorded as susceptible to rinderpest and they are in constant danger of exter-

mination in countries where epizootics occur. They live in fairly open country and often in close contact with cattle, whilst they further cover large distances and must be considered as an important means of spread. The author (1933) worked with a strain of virus recovered from an eland and recorded that passage through some 20 cattle demonstrated a normal virulence and death from rinderpest in each case.

The Pig.

These include the bush-pig (*Potamochoerus* sp.), warthog (*Phacochoerus* sp.) and giant forest hog (*Hylochoerus* sp.). They are all highly susceptible but on account of their local distribution do not carry the disease long distances, but owing to the ubiquitous distribution of the bush-pig this animal is a constant menace. In forest areas they are responsible for keeping the virus alive for long periods with the ever present danger of causing sporadic outbreaks in cattle and starting the disease in buffalo. Allan (1930) reports that the mortality in cattle infected from wild pig showed a marked decline and whilst the usual mortality was from 80 per cent. to 90 per cent. herds known to have been infected directly from pig lost 40 per cent. to 47 per cent.

Poulton (1914) experimentally infected several wild pig and proved their susceptibility to rinderpest in Uganda.

Recently a young bush-pig was infected at this Laboratory with known bovine virus and showed typical buccal and intestinal lesions and died on the eighth day following inoculation. Spleen emulsion was inoculated into six susceptible bovines; they all reacted and three died. This is a much lower mortality than is generally experienced in cattle inoculated with the same bovine virus at the Laboratory and records show that only two recovered out of 17 in which the disease was allowed to run its course. The results can only be taken as highly suggestive of a fall in virulence after passage through the pig but they accord with field experience.

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STUDIES IN ANIMAL TRYPANOSOMIASES

V.—Some Disturbances of the Host's Carbohydrate Metabolism induced by *Trypanosoma congolense* and *Trypanosoma brucei*.

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Many investigators have sought to determine the mechanism by means of which the parasites cause injury and death of hosts infected with trypanosomes. One result of this search has been to focus attention on the blood changes during the disease and all previous work indicates that there is some disturbance of the host's carbohydrate metabolism during infection. In fact, most work on the blood chemistry of trypanosomiasis has centred round this disturbance, and some have considered that this interference with the carbohydrate metabolism is partly or even completely responsible for the death of the infected animal.

The early importance attached to the carbohydrate metabolism in trypanosomiasis infections was the result of *in vitro* investigations supported by work on infected animals. The earliest observations dealing with this problem appear to be those of Biot, Biot and Richard (1911) and Fleig (1911), who showed respectively that the length of time *T. lewisi* and *T. brucei* survived in citrated blood was prolonged by the addition of glucose. Schern (1911) then demonstrated that *T. equiperdum* which had become motionless in citrated rat blood regained their motility if fresh blood or liver emulsion were added. Schern continued this work after the war and concluded that the active constituent was glucose. Yorke *et alia* (1929), Geiger *et alia* (1930), Regendanz (1930) and Von Brandt *et alia* (1932) have between them conclusively demonstrated that ample glucose is necessary for the continued existence *in vitro* of *T. rhodesiense, T. evansi, T. congolense, T. brucei* and *T. gambiense,* and it is now the established practice to add sugar to media for the *in vitro* cultivation of pathogenic trypanosomes. This work conclusively shows that trypanosomes have a very active metabolism and consume relatively large amounts of sugar.

There is an even larger volume of work on animal investigations. Schern (1925) and Fenyvessy (1926) found that as trypanosome numbers increased in infected rats, there was a diminution of blood sugar. Schern considered that during infection the blood sugar and liver reserves became depleted and that the hypoglycæmia with its sequelæ were largely contributory to the death of the host. Schern (1928) also found *T. equinum* infections of rabbits and a horse were accompanied at times by hypo- and at other times by hyperglycæmia. Scheff (1928) confirmed the work of Fenyvessy (1927) that there is an increased consumption of oxygen during trypanosome infections in the rat. Scheff (1928, 1932) concluded that the parasites deprived the host of sugar and oxygen and that hypoglycæmia

followed as a result of damage to the liver through functional overwork. The increased demand for oxygen together with the diminished number of red corpuscles then led to a state of internal asphyxiation, which was followed by an acidosis that still further reduced the reactive capacity of the host.

Many others have found a hypoglycæmia in infected animals but they did not regard it as contributing largely to the death of the host. Cordier (1927) and Savino (1927) both reported an irregular hypoglycæmia unrelated to the number of parasites in the blood. The former found that neither glucose nor insulin had any effect on the course of the infection, whilst the latter reported that the changes in blood sugar level induced by these substances did not parallel the changes in the number of circulating parasites.

Regendanz and Tropp (1927) found that a hypoglycæmia developed as infection progressed but, unlike Schern (1932) and Scheff (1928), they maintained that there was still enough glycogen in the liver at death to relieve, at least temporarily, the observed hypoglycæmia. These observers believed that injury and death of the host were due to toxins liberated by disintegrating trypanosomes. Since they only found a hypoglycæmia in the terminal stages of infection, they assumed that this was caused by a depressive action on the glycogenolytic mechanism by the liberated trypanotoxin and death was not due to a shortage of blood sugar. Dubois and Bouckaert (1927) and Bruynoghe *et alia* (1927) also concluded that there is enough glycogen in the liver during the marked terminal hypoglycæmia to maintain a normal blood sugar level, and they also postulated the production by the trypanosomes of a substance which slows down or prevents liver glycogen hydrolysis. Zotta and Radocovici (1929 *a, b*) also supported this hypothesis but pointed out that the terminal hypoglycæmia was not specific.

Kligler *et alia* (1928 and 1929) and Geiger *et alia* (1930) recorded a progressive increase in the blood lactic acid as the infection developed. They suggested that lactic acid was produced too rapidly for complete removal to take place, and that the resulting accumulation lowered the alkali reserve and interfered with the oxidative processes. A vicious circle would then develop and result in the internal asphyxiation of the host. That the acidosis was very important they proved by the life-prolonging action of injected bicarbonate (in spite of injury due to excess of $NaHCO_3$). Krijgsman (1934) also reported a terminal hypoglycæmia and a progressive increase in blood lactic acid, which led to an exhaustion of the alkali reserve in the terminal stages of an infection.

Linton (1929 and 1930 *a, b*) showed that, with highly pathogenic *T. equiperdum*, the blood of infected rats showed a marked premortal hypoglycæmia, whilst the CO_2-capacity was lowered even in the early stages of the infection. With non-pathogenic *T. lewisi* no hypoglycæmia nor lowered alkali reserve could be demonstrated. Later Linton and Poindexter (1931), from a study of the effects of induced

acidosis on *T. lewisi* infections, suggested that acidosis interferes with the host's defence mechanism. All the above observations have been made on small animals and it is interesting, therefore, to compare the findings when large domestic animals are examined. So far little has been done with these larger animals but Tubangui and Yutuc (1931) showed that a marked terminal hypoglycæmia developed in *T. evansi* infections of cats, dogs and horses. Randall (1935) working on controlled *T. evansi* infections in horses showed that blood sugar and CO_2-capacity were low at crises but returned to normal in the recovery phase. He concluded that the end-products of glucose consumption caused an un-compensated acidosis and depletion of the alkali reserve, whilst the concomitant anæmia led to anoxæmia. Jones (1933) could not detect any hypoglycæmia in trypanosome infected cattle in Uganda but his observations were made on animals selected at random and probably did not include animals in the terminal stages.

From this previous work it appears that three hypotheses have been put forward to account for the death of the host in trypanosome infections. They are (1) the hypoglycæmia theory, (2) the toxin theory, and (3) the theory of internal asphyxiation. The first, which postulates that death results from the exhaustion of liver glycogen and shortage of blood sugar, has been proved untenable by later workers. The toxin theory suffers from lack of direct evidence, though the negative results obtained when massive doses of disintegrating trypanosomes are infected cannot be taken as proof of the untenability of this hypothesis. Recent workers favour the theory of internal asphyxiation but, as will be shown later, this also is not wholly satisfactory.

Experimental.

The object of this investigation was to determine to what extent the above changes occur during *T. congolense* and *T. brucei* infections of farm livestock in Tanganyika. For the *T. congolense* work nine infected and two control oxen were examined throughout the course of the infections, together with two sheep and one donkey. With *T. brucei,* three infected and two control sheep, and one infected and one control donkey were examined. These examinations were made alongside the other blood studies already reported upon (French 1938, *a, b, c, d*) and the full details of the experimental animals and their rations have already been published (French and Hornby 1934, 1935).

Results.

(A) *Trypanosoma congolense Infections.*

Changes in the Sugar, CO_2-capacity and Lactic acid values of Cattle Bloods.

Because the anæmia of this disease affords the best means of following the course of an infection, all the results are tabulated alongside the red cell counts in Tables I to IV.

The blood changes observed can be divided into groups according to the nature and severity of the infections. In the cases of infection with a mild strain of trypanosome, with the oxen receiving a good ration, and where the animals recovered spontaneously, only very small changes were observed (Table III, oxen 3046 and 2225). The sugar values recorded fluctuated around normality with no hypoglycæmic tendencies and the lactic acid behaved similarly. A slight lowering of the alkali-reserve was associated with the lowest red cell counts, but these lower values were still within physiological limits of variation.

TABLE I.

SUGAR, CO_2-CAPACITY, AND LACTIC ACID VALUES OF OX BLOOD DURING *T. congolense* INFECTION ON A SUB-MAINTENANCE HAY RATION.

Ox 3143 (Infected).

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*	*Lactic Acid (mgm./100 ml.).*
0	10·0	63·2	65·0	9·0
2	7·7	54·5	55·9	5·4
4	6·1	66·2	56·6	13·2
6	4·6	66·2	58·9	5·9
8	4·2	56·4	—	9·9
10	3·5	67·9	—	—
13	3·5	63·5	50·7	14·9
15	3·5	64·6	55·1	26·8
19	3·6	61·2	50·6	24·0
20*	3·6	48·5	45·1	39·0
22	3·7	59·7	61·5	20·7
24	4·2	—	—	—
26	5·2	59·8	62·1	15·9
31	6·4	63·2	59·7	12·3
35	6·8	59·9	60·8	17·3
40	6·9	62·9	—	—
44	7·4	67·8	63·1	14·5

* Crisis.

Ox 2134 (Control).

0	9·5	68·3	64·3	10·1
4	8·6	72·9	66·0	13·9
8	8·8	71·2	63·8	14·4
13	7·9	69·5	70·2	8·9
19	7·1	61·4	69·7	11·9
22	7·1	—	67·3	18·6
25	7·5	63·1	60·6	14·8
31	8·1	64·5	67·8	11·1

In contrast with these cases are those in which the infection ended fatally, or in which a sharp crisis developed with the animal recovering only after treatment. In these cases the blood changes followed similar courses irrespective of the plane of nutrition. The fatal cases of oxen 2855, 3730, and 3622 showed only a terminal hypoglycæmia, but this did not develop progressively from the point of inoculation. The CO_2-capacity fell somewhat when the parasites were circulating in the blood, and this was followed by a further decrease with the onset of the terminal hypoglycæmia. The lactic acid increased in the later stages.

Ox 3143, who recovered without treatment after reaching a very distressing state, can be included with ox 2386 and 2864, who recovered only after antimony treatment. In these three oxen the blood changes up to crisis were exactly as for fatal cases. After crisis or after drug treatment had commenced, there was a return to normal of the three constituents under observation.

Ox 2785 (Table II) suffered from a chronic infection which developed into a long period of premunition before recovery was possible. Blood sugar values were normal and although the lactic acid increased and the CO_2-capacity diminished somewhat at crisis these values values remained within the normal range of variation.

TABLE II.

SUGAR, CO_2-CAPACITY, AND LACTIC ACID VALUES OF OX BLOOD DURING *T. congolense* INFECTIONS ON A MAINTENANCE HAY RATION.

Ox 2785 (Infected).

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*	*Lactic Acid (mgm./100 ml.).*
0	9·1	67·4	64·9	9·9
2	8·0	59·6	57·2	15·4
4	7·2	75·7	55·9	13·2
6	6·7	66·2	59·4	13·6
8	5·6	69·5	—	6·6
10	5·6	74·5	—	16·9
13	5·3	61·2	55·5	17·2
15	4·8	68·9	58·9	15·9
19	5·0	62·9	55·8	21·5
22	4·8	—	67·3	26·3
25*	5·4	63·1	53·7	21·4
31	5·2	71·4	51·8	19·9
35	5·8	68·7	63·3	18·3
40	5·8	67·8	—	—
44	6·1	70·6	67·3	16·0

* Crisis.

Ox 2863 (Control).

0	9·6	68·7	65·9	12·4
4	8·2	75·6	63·8	9·5
8	9·3	79·5	—	14·0
13	7·7	68·4	73·6	15·2
19	7·9	56·3	68·6	11·2
25	8·0	67·4	67·3	11·2
31	7·0	69·1	65·4	15·0
35	—	63·2	62·9	12·4
40	6·9	71·8	—	—
44	7·2	66·3	63·4	11·2

Ox 2855 (Infected).

0	9·0	63·8	61·1	10·8
5	6·8	71·5	45·6	14·9
9	4·1	63·8	48·3	15·7
12*	2·1	45·6	38·7	32·6
16	3·9	—	—	—

Died 4 days later.

* Crisis—given Antimosan.

Changes in the Sugar, CO_2-capacity and Lactic Acid values of Sheep Bloods.

Only two infected sheep were examined (Table VI), one on a good and the other on a poor ration, but the blood changes were the same for both sheep and both infections ended fatally. A terminal hypoglycæmia was observed, whilst the lactic acid values increased in the pre-mortal periods. As with the fatal cases in cattle, the CO_2-capacity fell to a lower level when parasites were demonstrable in the blood, but this initial drop was greater than was found for oxen, and the value then fell slowly until death.

TABLE III.

SUGAR, CO_2-CAPACITY, AND LACTIC ACID VALUES OF OX BLOOD DURING *T. congolense* INFECTIONS ON A GOOD RATION OF HAY AND COTTONSEED.

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*	*Lactic Acid (mgm./100 ml.).*
		Ox 3046 (Infected).		
0	10·3	72·9	63·8	13·8
2	6·6	61·2	—	11·8
4	6·4	74·6	64·5	—
6	5·6	71·4	53·4	15·4
10	5·7	68·1	52·9	12·2
15*	5·6	67·9	52·2	17·2
18	7·3	63·2	63·2	12·0
21	7·0	72·7	64·9	15·4
24	8·3	74·3	64·1	17·2
27	9·2	63·2	65·8	13·6
30	9·6	69·8	68·7	14·3
33	10·2	67·8	—	13·8
		Ox 2225 (Infected).		
0	9·6	69·5	62·8	12·8
2	7·9	56·3	—	10·0
4	6·4	66·9	—	—
6	5·5	63·9	62·8	15·4
10	5·3	64·7	51·8	13·8
15	4·3	63·1	54·9	11·0
18*	4·8	54·6	59·3	14·1
21	5·7	71·8	56·6	17·2
24	5·0	69·8	63·1	12·1
27	5·7	64·2	65·5	10·9
30	6·1	65·7	64·9	14·1
33	7·4	67·8	63·8	13·8
		* Crisis.		
		Ox 2864 (Infected).		
0	9·2	69·3	61·1	13·1
5	4·4	69·9	56·0	15·3
7	3·3	63·9	50·7	14·8
9	2·4	57·6	40·7	25·6
9½*	2·3	47·6	35·6	25·7
10	3·0	—	—	—
12	4·3	68·4	58·6	19·8
14	4·6	67·2	62·8	18·7
18	5·7	70·4	70·6	17·9

* Crisis—treated with Antimosan.

Changes in the Sugar and CO_2-capacity values of Donkey Blood.

Table VII shows that following the inoculation of a donkey with *T. congolense* there was very little development of anæmia, and no lowering of the blood sugar and CO_2-capacity levels for six weeks. During the eighth week after inoculation *T. brucei* suddenly appeared in the blood of this animal, and this was immediately followed by rapid anæmia. The blood sugar and CO_2-capacity values fell slowly as the animal approached death.

(B) *Trypanosoma brucei infections.*

Changes in the Sugar, CO_2-capacity and Lactic Acid values of Sheep Bloods.

Three infected sheep were compared (Table VIII) with two controls, and two planes of nutrition were employed. In all three infected animals the disease ran an acute course and ended fatally. The premortal hypoglycæmia was not so marked as for *T. congolense* infections, but was present in two cases, although sheep Imani did not show any lowering of his blood sugar level. The alkali reserve steadily decreased in all three infected animals, whilst the lactic acid values steadily increased.

Changes in Sugar, CO_2-capacity and Lactic Acid values in Donkey Bloods.

In Table IX the blood changes of a control and a *T. brucei* infected donkey are compared. A marked anæmia developed rapidly, and the CO_2-capacity fell to a lower level as soon as parasites appeared in the blood and a further fall took place as death approached. An abrupt agonal hypoglycæmia was recorded and there was no lowering of the blood sugar level in the early stages of the infection.

TABLE IV.
SUGAR, CO_2-CAPACITY, AND LACTIC ACID VALUES OF OX BLOOD DURING *T. congolense* INFECTIONS ON AN *ad lib.* HAY RATION.

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*	*Lactic Acid (mgm./100 ml.).*
		Ox 2386 (Infected).		
0	8·9	69·1	62·8	14·2
3	6·6	66·5	59·7	12·9
6	4·9	65·7	—	14·8
9	4·6	75·1	47·9	17·2
14	3·6	68·3	52·1	23·9
17	3·8	—	52·2	25·6
19*	3·4	56·7	54·1	28·3
20	3·6	51·0	61·1	20·1
25	4·5	72·7	63·3	17·6
31	5·0	68·2	64·9	16·9
34	4·9	65·7	61·1	15·1
39	5·4	72·6	62·1	13·8
		* Crisis.		
		Ox 3730 (Infected).		
0	8·8	71·0	65·3	14·2
3	7·3	72·0	55·9	11·8
6	4·5	62·8	—	15·9
9	3·2	47·1	27·7	32·9

Died 3 days later.

Table V.

Sugar, CO_2-capacity, and Lactic Acid Values of Ox Blood during *T. congolense* Infection on a good Mixed Diet.

Ox 3622 (Infected).

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*	*Lactic Acid (mgm./100 ml.).*
0	8·7	69·3	64·9	12·8
5	4·4	74·6	56·0	14·6
7	3·5	65·4	57·3	17·9
9	2·8	68·3	53·4	17·9
11	2·2	52·6	47·2	26·2
12	1·9	48·3	44·8	36·3

Died 1 day later.

Table VI.

Sugar, CO_2-capacity, and Lactic Acid Values of Sheep Blood during *T. congolense* Infections.

Sheep W (Infected on poor hay ration).

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*	*Lactic Acid (mgm./100 ml.).*
0	14·8	61·2	59·1	15·2
2	11·2	59·6	53·2	17·7
4	9·8	65·1	46·3	12·9
6	6·2	68·9	41·2	15·9
8	6·4	76·2	38·3	19·2
11	5·3	55·3	38·5	28·3

Died 7 days later.

Sheep M (Infected on good ration).

0	15·1	60·6	60·6	14·3
2	11·2	54·5	49·5	17·6
4	8·8	68·3	41·6	19·2
6	8·3	74·3	48·4	15·8
8	6·9	76·2	42·4	12·2
11	6·2	66·3	39·6	20·0
17	5·6	51·1	42·4	22·9

Died 9 days later.

Table VII.

Sugar and CO_2-capacity Values of Donley Blood during Infection with *T. concolense* and later with *T. brucei.*

Donkey 17 (Infected on an ad lib. hay ration).

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*
0	7·3	74·9	68·4
2	—	73·1	61·8
4	7·6	74·6	60·3
6	6·7	76·9	64·5
8		*T. brucei* suddenly appeared in blood slides	
9	3·9	63·8	58·7
10	2·7	51·6	55·9

Destroyed 1 week later.

TABLE VIII.

SUGAR, CO_2-CAPACITY, AND LACTIC ACID VALUES OF SHEEP BLOOD DURING *T. brucei* INFECTIONS.

Sheep Umoyo (Infected on poor hay ration).

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*	*Lactic Acid (mgm./100 ml.).*
0	15·2	71·7	68·6	15·7
3	10·8	64·6	58·7	22·2
6	9·5	58·0	50·9	33·4
		Died 1 day later.		
		Sheep Dunia (Control on poor hay ration)		
0	12·8	70·7	67·5	13·7
3	15·3	76·9	63·1	9·1
6	14·2	74·4	62·8	14·8
		Sheep Imani (Infected on poor hay ration).		
0	10·0	68·9	65·6	17·1
2	7·8	66·5	55·8	17·2
5	6·5	68·2	43·7	35·2
		Died 6 days later.		
		Sheep Tumbo (Infected on good ration).		
0	12·3	81·7	64·8	13·8
3	8·8	71·7	59·3	12·5
6	8·8	66·8	61·4	19·0
9	5·0	61·7	44·4	22·9
		Died 2 weeks later.		
		Sheep Majani (Control on good ration).		
0	12·5	71·7	59·3	15·2
3	12·2	68·8	63·9	10·2
6	12·6	70·1	65·1	15·0
9	12·9	75·4	62·8	16·1

TABLE IX.

SUGAR AND CO_2-CAPACITY VALUES OF DONKEY BLOOD DURING *T. brucei* INFECTION ON AN *ad lib.* HAY RATION.

Donkey 3 (Infected).

Weeks.	*Red Cells (millions).*	*Blood Sugar (mgm./100 ml.).*	*CO_2-capacity (vols. %).*
0	7·9	73·1	63·1
2	—	74·9	55·7
4	4·3	71·9	50·3
6	2·3	70·8	50·4
6½	2·1	37·5	39·0
		Killed *in extremis* after the last bleeding.	
		Donkey 22 (Control).	
0	7·1	71·9	64·5
2	7·8	70·6	65·3
4	7·5	74·6	62·7

Discussion.

It is advisable, first, to draw attention to the differences between trypanosome infections in small laboratory animals, such as have been used by the majority of investigators, and those in the larger farm animals. In rats trypanosomes multiply rapidly and at death the infection may be so "Swarming" that there are as many parasites as there are red blood corpuscles. In guinea pigs multiplication proceeds less regularly and to a less extent, but at death the blood is also swarming with parasites. The number of parasites in the blood of the larger farm animals, even at death, is relatively small and does not approach the heavy infections found in the small animals. In fact it is often difficult to find *T. brucei* in blood smears from cattle and sheep long after the animals are showing obvious clinical symptoms of the disease. However, in spite of these big differences in the number of parasites in the blood, the ultimate result is the same—death or severe injury to the host.

The mere presence of trypanosomes in the body cannot account for the toxic manifestations of pathogenic species. In rats, when equally large numbers of the parasites are present, *T. lewisi* does not cause the marked metabolic disturbances found during infections with pathogenic *T. equiperdum* (Linton 1929 and 1930 *a*, *b*). Similarly *T. melophagium* in sheep and *T. theileri* in cattle, although they are most common, are but rarely pathogenic.

The early hypothesis that death from trypanosomiasis is due to an exhaustion of liver glycogen and a shortage of blood sugar is of little significance in the diseases of large animals. Owing to the large carbohydrate intake of cattle, sheep and donkeys it is most unlikely that the small number of trypanosomes present in the body could, by their direct consumption of glucose, seriously affect the blood sugar level. Certainly they could not cause by this means an exhaustion of the carbohydrate reserves of the host. Even in small animals where relatively many more parasites are active, a number of workers have shown that there is at death sufficient glycogen in the liver to relieve, at least temporarily, the observed hypoglycæmia.

The internal asphyxiation hypothesis is also not sufficiently satisfactory to account for the death of large animals. In spite of their active metabolism, and possibly because their enzymatic peculiarity prevents pathogenic trypanosomes from oxidising carbohydrate material completely, the lactic acid production of the parasites could not reach a sufficiently high pitch to alter the level in the bloods of large animals. Lactic acid, whether injected or developing from tissue metabolism, is rapidly removed from the blood stream in a normal animal and converted into liver glycogen.

Supporters of the asphyxiation theory also claim that trypanosomes interfere with the normal oxidative processes of the body and so cause the host to produce lactic acid at a higher rate. The few parasites in large animals cannot consume oxygen at a sufficient rate to deprive the host of an adequate amount. Further, because of their

small numbers, it is unlikely that they can impede the normal oxygen transport to the tissues by simple mechanical obstruction in the capillaries. By reducing the number of red blood corpuscles, trypanosomes are still further supposed to interfere with the transport of oxygen in the host. A reduction of the red cells does take place but, in the early stages of the infection, these have not fallen sufficiently for the host to be unable to compensate for this lowered oxygen carrying power by increasing its circulation and respiration rates. The results of several previous investigators, as well as those of the present experiments, indicate that an acidosis develops as soon as the incubation period is over. This lowering of the alkali reserve thus commences long before the anæmia has outstripped the compensatory mechanism of the host for maintaining an adequate supply of oxygen to all the tissues. The accumulation of lactic acid which leads, in the later stages of an infection, to an exhaustion of the alkali reserve will, when coupled with the anoxia resulting from the anæmic state of the blood, contribute to the death of the host, but the asphyxiation hypothesis does not account adequately for the mechanisms bringing about the anæmia and acidosis.

Certain workers have been content to dispose of the toxin theory because massive injections of dead and disintegrating trypanosomes failed to produce a toxæmia in rats. Similarly the injection of serum obtained from heavily infected rats just before death also produced no ill-effects. Mere destruction of trypanosomes, however, cannot account for the toxic symptoms, otherwise they would be present during the destruction of non-pathogenic species. Also the injection of infected animals with trypanocidal drugs would be followed at first by increased metabolic distrubances whereas it is known that the metabolism returns to normal extremely quickly.

There has been a tendency on the part of a number of investigators to focus their attention too sharply on the carbohydrate metabolic disturbances and to leave out of account other characteristic symptoms of trypanosomiasis. The anæmia which accompanies infections in large animals can be accounted for by assuming that the trypanosomes either (1) remove from the system one or more of the essential building stones required for the synthesis of hæmoglobin and the red blood corpuscles, or (2) elaborate a hæmatopoietic inhibitor. The evidence of Hornby (1933) and of French and Hornby (1934, 1935), together with later unpublished data, suggest that the second hypothesis is the more likely. If this is not so it becomes very difficult to account for the less severe anæmia which develops when an ox relapses after treatment of an acute infection. In such cases the infection level may be the same as before treatment yet the anæmia and metabolic disturbances are less severe and the animal suffers a chronic infection which passes off into a stage of premunition prior to complete recovery. It is assumed that actively metabolising pathogenic trypanosomes produce a toxin which inhibits red cell regeneration, but that as a result of a previous infection, antibody

formation against this toxin develops more quickly and the infection is then controlled. Previous work at Mpwapwa has also shown that during the wasting of body tissues during trypanosomiasis there is an increased endogenous protein catabolism similar to the "toxic destruction of protein" found in other febrile infections. The degeneration of parenchymatous tissues that is often evident in cases of trypanosomiasis is consistent with the idea of a trypanotoxin elaboration as, also, is the fever.

If it is permissible to assume the production of a toxin, whether produced during the metabolism of the parasite or elaborated as a result of reactions between the trypanosomes and its host, then many phases of the carbohydrate disturbances can be explained. Corkill, *et alia* (1932, 1934 *a*, *b*) have shown that diphtheria toxæmia in the rabbit interferes with the power of the liver to form glycogen from lactate. If a similar interference occurs in trypanosomiasis (this point is now under investigation), blood lactic acid, whether produced by the trypanosomes or in the host's normal carbohydrate metabolism, would then be free to deplete the host's alkali reserve. This mechanism would explain both the early development of acidosis and the increase in blood lactic acid in the terminal stages. The blood sugar would remain normal because such a toxæmia would not interfere with the glycogenolytic function of the liver. When the development of anæmia has reached the stage beyond which the host cannot compensate for the lowered oxygen carrying capacity of its blood, the effects of anoxia will be added to those of exhaustion of alkali reserve and the overpowering of the host's defence mechanisms, and death will follow quickly. It is in this last stage when shortage of oxygen has caused degenerative changes in the tissues that the liver breaks down through functional over-work and its glycogenolytic activity is impaired. The premortal hypoglycæmia would then follow as a direct consequence.

Summary.

(1) There is no gradual development of hypoglycæmia during *T. congolense* and *T. brucei* infections of large animals, but a sharp fall in the blood sugar level occurs shortly before death or crisis. In animals that are treated or recover without therapeutic aid, the blood sugar level returns to normal immediately crisis is passed.

(2) The CO_2-capacity appears to fall as soon as the incubation period is over. A further drop usually occurs towards the end of the infection. It can be considered that an acidosis exists during infection and then becomes more pronounced as death approaches.

(3) Blood lactic acid values increase in the premortal stages and near crisis.

(4) A marked carbohydrate disturbance is caused in the host by pathogenic trypanosomes, but its mechanism has not yet been elucidated clearly.

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THE TOXICITY OF *BR. ABORTUS* FOR MICE

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It has been known for many years that the intraperitoneal inoculation of large numbers of *Brucella* organisms may cause death in mice or rats within a short space of time. Thus Holth (1911) showed that death often followed in one to four days when mice or rats were inoculated intraperitoneally with 0·5 c.c. of serum broth culture of *Br. abortus*. Furthermore, he found that immune serum gave considerable protection against the toxic effect of the cultures: the protective action could be demonstrated either by inoculating serum some time before the culture or by the inoculation of sensitised organisms. Holth also showed that animals could be protected against the toxic effect of *Br. abortus* by vaccination with either heat killed cultures or culture filtrates.

These findings were confirmed by Zwick and Zeller (1913) and by Weigman (1931).

Weigman (1931) also showed that immunity to the toxic effect was not species-specific, since mice vaccinated with either *Br. abortus* or *Br. melitensis* showed no difference in their resistance to a subsequent inoculation of either organism. Similar findings are recorded by Helms (1932) and by Singer-Brooks (1937). The latter found that mice inoculated with a heat-killed vaccine were protected against the intraperitoneal inoculation of 300 million *Br. suis* which caused death in control animals within four days, and that mice vaccinated with the porcine strain were immune to the rapidly fatal toxic action of either *Br. abortus* or *Br. melitensis*.

Animals immune to the toxic effects of *Brucella* are not however immune to the more chronic type of disease which in fact develops in the vaccinated animals, as has been shown by Holth (1911) and Singer-Brooks (1937).

The literature contains few references to the degrees of toxicity of different strains of *Brucella*. Holth (1911) suggested that the toxicity of different strains of *Br. abortus* might vary but no experiments on this subject were recorded by him. Singer-Brooks found that *Br. melitensis* and *Br. suis* were more toxic for mice than *Br. abortus*. A further suggestion of variability in toxicity may be gathered from an experiment reported by Ber (1933) in which two CO_2 sensitive strains of *Br. abortus* were inoculated intraperitoneally each into two mice while a third aerobic and rough strain was similarly inoculated into a third group of two mice. The animals receiving the CO_2 sensitive strains died within 48 hours whereas those inoculated with the rough strain survived. Singer-Brook's experiments therefore suggest that the more virulent melitensis and porcine species of *Brucella* are more toxic than the less virulent bovine species and Ber's work indicated that recently isolated CO_2 sensitive

Br. abortus strains are more toxic than aerobic rough strains of this organism.

As we are interested in estimations of the virulence of different strains of *Br. abortus* preliminary experiments were undertaken to see whether strains showing different degrees of virulence for guinea-pigs also showed different degrees of toxicity for mice. Experiments were also undertaken in an endeavour to explain the cause of the toxicity. In our work suspensions for inoculation have been prepared from 2-day liver agar growths. The growth has been washed off with saline and either concentrated or diluted to the required density and 0·5 c.c. amounts have then been inoculated intraperitoneally. This has been the general procedure throughout; any variation is noted in the text.

Mice inoculated with suitable doses generally die within four days; only rarely do toxæmic deaths occur after that time. Experiments of this nature have therefore been terminated after four days.

At *post-mortem* examination the most constant finding was a dilatation of the small intestine with fluid and gas. In a small proportion of the mice localised areas of necrosis or diffuse necrosis of the liver were found; occasionally fluid was present in the peritoneal cavity and sometimes there was a fibrinous deposit on the liver and spleen or even adhesions. The thoracic cavity and its contents were invariably normal in appearance.

The Toxicity of Strains of Different Degrees of Virulence for Guinea-pigs.

With virulent strains it was found that the average lethal dose was about $1{,}000 \times 10^6$ viable organisms, and this is approximately the same whether given intraperitoneally or intravenously; intraperitoneal inoculations were therefore used throughout this work. Mice are highly resistant to subcutaneous inoculation. There is generally abscessation and necrosis of the overlying skin but the animals can withstand at least 20 intraperitoneal lethal doses and they never die suddenly from toxæmia.

There is however a very distinct difference in the sizes of the average lethal dose of virulent and non-virulent strains. Table I shows the results of one experiment designed to test this point. Suspensions were prepared as outlined above and very carefully standardised (by the Brown opacity standards) to contain approximately $16{,}000 \times 10^6$ organisms per c.c. Dilutions were made as indicated, a separate pipette being used for each step in the work. 0·5 c.c. amounts of each dilution were inoculated intraperitoneally into ten mice, which were observed for four days. Strains 817 and 544 were fully virulent and CO_2 sensitive strains and both strains caused infection in guinea-pigs when less than 100 organisms were inoculated subcutaneously; given by the eye about 1,000 organisms infected. Infected guinea-pigs showed gross enlargement of the spleen and remained infected for months. In other words these two strains were of ordinary virulence. Strain 45 is an old laboratory strain and may

be regarded as completely non-virulent for guinea-pigs. Strain AA was isolated a few years ago and is relatively non-virulent for guinea-pigs, causing no splenic enlargement and only a transient infection.

Table II shows the results of another experiment of the same sort, in which, however, only five mice were used for each dose.

TABLE I.

SHOWING THE TOXICITY OF VARIOUS STRAINS OF *Br. abortus* FOR MICE.

Strain.	*Type.*	*Number of Organisms Inoculated. (In thousand millions.)* 8	4	2	1	0·5
817	Virulent	7	8	6	6	0
544	„	8	4	4	1	1
45	Non-virulent	1	0	0	0	0
AA	„	1	0	0	0	0

Figs. = Number dying out of 10 inoculated.

TABLE II.

SHOWING THE TOXICITY OF VARIOUS STRAINS OF *Br. abortus* FOR MICE.

Strain.	*Type.*	*Number of Organisms Inoculated. (In thousand millions.)* 16	8	4	2	1
746	Virulent	4	5	4	3	0
544	„	5	5	5	0	0
45	Non-virulent	3	0	0	0	0

Figs. = Number dying out of 5 inoculated.

These results clearly indicate the difference in the toxicity for mice between virulent and non-virulent strains.

By using carefully standardised doses it may even be possible to demonstrate grades of virulence, and we have shown this with three strains 45, 45 (6) and 45 (20). Strain 45 cannot be isolated from guinea-pigs 14 days or so after the subcutaneous or intra-muscular inoculation of 10,000 × 10^6 viable organisms. This is the strain described by McEwen and Roberts (1936), McEwen (1937), McEwen and Priestley (1938) in their vaccination studies. Strains 45 (6) and 45 (20) have been derived from Strain 45 by guinea-pig passage and they now possess some degree of virulence for guinea-pigs. Neither Strain 45 (6) nor 45 (20) produces any splenic enlargement in guinea-pigs inoculated with 2,000 million organisms but when animals are inoculated with relatively small numbers of either strain *Brucella* may be recovered from their spleens in a percentage of cases six or more weeks later. With Strain 45 (20) the percentage of guinea-pigs showing infected spleens is greater than with Strain 45 (6), the former strain being the more virulent.

In Table III are shown the results of testing the toxicity of these strains for mice. Groups of ten mice were inoculated intraperitoneally with the doses indicated, and the deaths noted.

Table III.

Showing the Toxicity of Strains 45, 45 (6) and 45 (20) for Mice.

Strain.	*Number of Organisms Inoculated. (In thousand millions.)*				
	61·5	30·7	15·4	7·7	3·8
45	10	8	5	2	0
45 (6)	10	9	8	1	1
45 (20)	10	10	10	8	7

Figs. = Numbers dying out of 10 inoculated.

The Cause of Toxicity.

Holth's original observation that the toxic factor appeared to be heat-labile was confirmed.

Suspensions of virulent organisms containing at least ten lethal doses when boiled for a few minutes fail to kill mice. This factor indeed appeared to be destroyed at 56° C. in about 20 minutes as is shown in Table IV.

Table IV.

Showing the Effect of a Temperature of 56° C. on the Toxicity of *Br. abortus*.

Minutes of Heating at 56° C.	*Numbers Inoculated.*	*Numbers Dying.*
0	4	4
5	4	4
10	4	2
15	4	3
20	4	0
25	4	0
30	4	0

This fact seemed to differentiate the toxic action of *Br. abortus* from that of say, *Bact. coli*, which is due to the heat-stable somatic antigen, moreover it showed that it was not due to a mere collection of foreign particles in the tissues.

As a result of this finding attempts were made to demonstrate something in the nature of an exotoxin or a toxic enzyme. Filtrates of broth cultures, and saline and distilled water extracts of the bacilli all proved to be non-toxic. Similarly all attempts to demonstrate some enzymic factor were unsuccessful; these attempts followed the lines laid down by Quastel and his co-workers (see Stephenson, 1930) and included attempts to kill the organism, without damaging its enzyme structure, by toluene, potassium permanganate, phenol, formalin, hydrogen-peroxide, and freezing and thawing.

Experiments with the freezing and thawing technique showed that *Br. abortus* was relatively resistant to this treatment. Suspensions of *Br. abortus* including virulent and non-virulent, CO_2 sensitive and aerobic strains, were immersed in a mixture of solid CO_2 and acetone for two to three minutes until frozen solid and then immediately transferred to a bath

at 37° C. This procedure was repeated continuously, sub-cultures being made at intervals; all the strains tested survived this treatment 80 times and many of them over 144.

Another observation by Holth, namely, that the toxic effects of *Br. abortus* were neutralised by immune serum was also confirmed.

In an experiment designed to test this point 30 mice were each inoculated intraperitoneally with 0·5 c.c. immune serum prepared in a rabbit, 30 received the same dose of normal rabbit serum (two of these died overnight), and 30 received saline; the next day all mice received an inoculum of living *Br. abortus*. The results are shown in Table V.

TABLE V.
SHOWING THE PROTECTIVE ACTION OF IMMUNE SERUM.

Primary Inoculation.	*Number of Mice Inoculated.*	*Number of Mice Dying.*
Immune serum	30	0
Normal serum	28	25
Saline	30	26

Continuing this work we found that the protective action of immune serum was specific, *i.e.*, an *abortus* serum protects against the toxic action of *Br. abortus* but not against *Bact. coli.*

That serum protection is due to ordinary somatic antibody and not to an antibody produced in response to some hypothetical and hidden antigen peculiar to virulent strains of *Br. abortus* is indicated by the fact that protection is equally pronounced with serum prepared from virulent and non-virulent organisms. This fact is illustrated in Table VI.

TABLE VI.
SHOWING THE PROTECTIVE ACTION OF SERA PREPARED BY INOCULATION OF VIRULENT AND NON-VIRULENT STRAINS OF *Br. abortus*.

Serum Inoculated.	*Titre.*	*Number of Mice Dying.*	*Number Surviving.*
Anti-virulent	1 : 1280	1	9
Anti-non-virulent ...	1 : 640	0	10
None (controls)... ...	—	10	0

A further proof that the protective action of immune serum is due to somatic antibody is to be found in the fact that smooth sera protect against smooth but not against rough strains. In an experiment testing this point 20 mice were inoculated intraperitoneally with 0·5 c.c. immune serum which had an agglutinin titre of 1 : 2560 when tested against the standard suspension of *Br. abortus;* 24 hours later ten of these mice together with ten controls were inoculated intraperitoneally with 0·5 c.c. of the smooth strain 817 (8 a.l.d.s), while the other ten mice together with another ten controls received 0·5 c.c. of the rough strain 45 (20) (2 a.l.d.s). As Table VII shows, all the control mice and all the mice receiving smooth serum and rough suspension died, whereas only one of those receiving serum and smooth suspension died.

TABLE VII.

SHOWING THE SPECIFICITY OF IMMUNE SERUM.

Material Inoculated.		*Numbers Inoculated.*	*Numbers Dying.*
Serum.	*Suspension.*		
Smooth	817 smooth	10	1
Smooth	45 (20) rough	10	10
None	817 smooth	10	10
None	45 (20) rough	10	10

Finally in this connection it should be noted that immune sera of the same titre are equally efficient whether prepared by the inoculation of living or dead organisms.

Since immune serum protects against the toxic action and further since there is apparently nothing in the antigenic structure of *Br. abortus* other than the heat-stable somatic antigens (see Priestley, 1938) we were led to suspect that perhaps, after all, it was these antigens which were responsible for the toxic action. This belief was supported by the finding that very large doses of dead organisms also killed mice. It was therefore argued, that, if virulent and non-virulent strains which did not differ appreciably in their antigenic structure could be found, the smallest number of dead organisms necessary to kill mice should be approximately the same in the two types even though there was a considerable difference when they were used living. Virulent organisms in the living state might be more toxic because of their ability to survive and presumably to multiply rapidly in the tissues, thus quickly producing enough somatic antigen to kill; that completely non-virulent strains do not multiply appreciably in the tisssues we have shown by direct cultural methods. The results of an experiment testing this hypothesis are shown in Table VIII. For this experiment heavy suspensions of strains 817 and 45 were prepared, and various dilutions made as indicated. A portion of each of the resultant suspensions was placed in a boiling water bath for one hour and cultural controls showed that the organisms were all killed by this treatment. The remaining portions were untreated. Groups of five mice were then each inoculated with 0·5 c.c. volumes of the various suspensions and the deaths recorded. The antigenic structure of these two strains was shown to be comparable by the usual cross-absorption tests.

The one or two odd deaths that occurred were probably due to some intercurrent infection, but to satisfy ourselves that the result was not accidental we repeated a part of the experiment. Groups of ten mice were inoculated with suspensions of strains 817 and 45 boiled for one hour. The results, shown in Table IX, confirmed the previous findings.

Finally we tested the effect of immune serum on dead organisms. Boiled organisms were mixed with an equal volume of immune rabbit serum and, after a short incubation, 1·0 c.c. of the mixture

TABLE VIII.

SHOWING THE TOXIC ACTION OF LIVING AND DEAD, VIRULENT AND AVIRULENT *Br. abortus*.

Strain.	*Type.*	*Numbers of Organisms Inoculated.* (*In thousand millions.*)							
		51·2	25·6	12·8	6·4	3·2	1·6	0·8	0·4
817 living	Virulent	5	5	5	5	5	5	4	4
817 dead	,,	5	3	2	0	1	0	0	1
45 living	Avirulent	5	4	5	1	1	2	0	0
45 dead	,,	4	2	1	0	0	0	0	0

Figs. = Numbers dying out of 5 inoculated.

TABLE IX.

SHOWING THE SIMILARITY BETWEEN THE TOXIC EFFECT OF LIVING AND DEAD, VIRULENT AND NON-VIRULENT *Br. abortus*.

Strain.	*Type.*	*Numbers of Organisms Inoculated.* (*In thousand millions.*)					
		98·3	49·2	24·6	12·3	6·1	3·1
817	Virulent	10	10	2	0	0	0
45	Avirulent	10	10	8	0	0	0

Figs. = Numbers dying out of 10 inoculated.

inoculated intraperitoneally into mice; as a control a mixture of boiled organisms and normal rabbit serum was similarly prepared and inoculated. Nine of the former and all of the latter groups died *i.e.*, there was little or no protection, although the average time to death was greater in the groups receiving immune serum ($2{\cdot}1 \pm 0{\cdot}23$ days, as compared with $1{\cdot}4 \pm 0{\cdot}16$ days). These results, we think, indicate that immune serum does not exert any marked neutralising effect on the toxin, such as one finds when mixtures of typical exotoxin and its antitoxin are prepared.

DISCUSSION.

These experiments indicate that the toxicity of *Br. abortus* for mice runs parallel with its degree of virulence and that the toxicity test might provide a rapid and relatively inexpensive means of estimating virulence. So far we have only worked with what may be regarded as fully virulent strains of *Br. abortus* and strains of definitely reduced virulence. It has been found that a difference in the toxicity of these types is readily shown and that degrees of toxicity among strains of definitely reduced virulence can be demonstrated. Should there be measurable degrees of virulence intermediate between the virulent and relatively non-virulent strains studied it is still a matter of speculation whether these may be demonstrable by any toxicity test. We do not think it likely that the testing of the toxicity for mice will furnish a more *sensitive* measurement of virulence than the inoculation of groups of guinea-pigs with graduated doses of culture. From other work to be published later it appears unlikely that, in the upper ranges, degrees of

virulence can be readily assessed, at least not without the use of an impractically large number of animals.

The cause of the toxicity is a matter for speculation. The available facts are not inconsistent with the belief that toxicity is due to either the whole or a part of the heat-stable somatic antigen. This is indicated by the facts that (*a*) the average lethal dose of both virulent and non-virulent strains are approximately the same when the organisms have been killed, and (*b*) passive protection against living organisms can be demonstrated by the inoculation of immune serum. It should be noted that immune serum is equally efficient whether it is prepared by the inoculation of living or dead organisms, or of virulent or non-virulent strains, *i.e.*, the antigen concerned is not peculiar to living organisms nor to toxic strains. Moreover the protective action is specific, *i.e.*, a smooth serum protects against smooth but not against rough strains.

On the other hand, if it be assumed that the toxicities of killed and living organisms have different causes, it may well be argued that there is a heat-labile toxic factor common to virulent strains and absent or only present in minute quantities in non-virulent strains. This is indicated by the fact that toxicity is related to virulence and not to smoothness or roughness, *e.g.*, the rough strain 45 (20) (see McEwen and Priestley, 1938) is more virulent and more toxic than the smooth strain 45 but less so than the smooth strains 544 and 817. If this explanation is to be tenable it must further be assumed that the toxic factor is non-antigenic, at least when incorporated within the bacterial body, since serum prepared from a highly toxic smooth strain does not protect against a less toxic rough strain.

At present it would seem unwise to be dogmatic about the cause of this toxicity.

Summary.

The intraperitoneal inoculation of mice with suitable doses of *Br. abortus* results in death in one to four days. There is a definite relationship between this toxicity for mice and virulence as judged by the ability to infect guinea-pigs, virulent strains being more toxic.

The cause of the toxicity has been discussed.

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"WHITE SCOUR" IN CALVES AND RELATED INFECTIONS

I.—The Significance of the Vitamin A Content of the Colostrum as a Predisposing Factor in the Causation of such Conditions.

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It has been computed by several authorities in this country that the death-rate in calf populations during the first ten days of life is very high—in some cases being over 25 per cent. Three conditions are mainly responsible for this high mortality, namely, white scour, navel-ill and joint-ill. It is rarely that one of these diseases is prevalent without the presence of one or both the others. The chief localised clinical features of these diseased conditions are illustrated in their names. White scour is characterised by the chalky-white colour of the fæces which before death may change to bloodstained mucus; navel-ill by a purulent inflammation of the navel; and joint-ill by a sero-fibrinous or purulent arthritis with enlargement of the affected joints, especially those of the hock and knee, with resultant lameness. It is now generally believed that there is a close relationship between these three conditions and the statement of Jensen (1913) that the chief infecting agents are various strains of *B. coli* has been upheld by several bacteriologists (Lovell and Hughes (1935), Miessner and Koser (1935), Sigmund (1934)), though other organisms have been shown to be present. The primary causes, therefore, of these diseases would appear to be facultative pathogenic bacteria which only become pathogenic under the influence of certain predisposing factors.

The non-specific nature of these infections has made it difficult to utilise the methods of treatment and prevention commonly adopted in the case of bacterial diseases—vaccines and sera having proved of little value. Research workers have therefore turned their attention to the nature of the predisposing factors in order to find a method of prevention. Smith and Little (1922*a*) by means of carefully controlled experiments clearly demonstrated that the colostrom of the mother played an important rôle in the prevention of white scour and allied conditions in calves. They showed that the death rate in colostrum-fed calves was very much lower in comparison with that of calves denied colostrum. They suggested that the calves deprived of colostrum lacked some factor which allowed intestinal bacteria to invade the body and multiply in the various organs. In a later paper Smith and Little (1922*b*) postulated that a sufficiency of the mother's antibodies passed to the calf via the colostrum and helped it in the first days of life to combat infection from miscellaneous bacteria, which are harmless later when the protective mechanisms of the calf have begun to operate. Unfortunately the data upon which this theory was based were open to criticism.

Until lately the work of Smith and Little was not continued and apart from bacteriological surveys calf mortality received but scant attention. Recently, however, several workers have shown that cows' colostrum as distinct from milk, possesses a very large amount of vitamin A, and it was considered possible that the essential constituent of the colostrum, which seemed to confer immmunity to white scour, might be vitamin A. Mellanby and Green's (1929) classical studies on vitamin A and the rôle it plays in resistance to infection and especially the use of the term "anti-infective vitamin" have led to much controversy as regards whether or not vitamin A is capable of being of anti-infective value. As a result of the histological research carried out on hypovitaminosis A scientific opinion now concedes that extreme depletion of vitamin A in the body predisposes to bacterial infection, since one of the most characteristic properties of vitamin A is its power of influencing the development of epithelial structure in the body. In the absence of vitamin A the cells of the alimentary and genito-urinary passage as well as those of certain other organs undergo gross changes which are of great importance since they are of such a nature as to lower surface penetrability to bacteria. Therefore, infections of the *B. coli* type are more likely to occur in animals suffering from a gross deficiency of this vitamin. The following experiments were carried out in an attempt to assess the value of the vitamin A content of the colostrum in producing immunity to the calf diseases described above.

Fourteen farms on which the calf mortality had been high in previous years were selected, and from February till May, 1937, colostrum samples were obtained from over 100 cows. Seven samples were obtained from each cow: the first was taken within a few hours of calving and the others at successive daily milkings. The vitamin A content of the samples was estimated and subsequently a record of the calf's health obtained. The cows were stall-fed on scientifically balanced rations. The majority of the cows belonged to attested herds and were therefore being kept under ideal hygienic conditions. The results obtained shed considerable light on the factors influencing the individual variation in the vitamin A content of the colostrum of cows kept under the same conditions of dieting and management and have already been reported and discussed from this point of view by the authors (Stewart and McCallum, 1938). The findings may be recapitulated as follows: the vitamin A content of the colostrum of 100 cows was found to range from 1,280 to 59 B.U. per 100 ml.; this wide range was not due to any differences in dietary, breed or date of calving in animals kept under the same conditions of mangement: the variation on individual farms was also found to be large, ranging in one case from 994 to 126 B.U. and in another from 760 to 130 B.U. per 100 ml.: the chief factor affecting the vitamin A content of the colostrum appeared to be the length of the non-lactating period between successive calvings: the vitamin A content of the colostrum fell very rapidly from the day of calving—

TABLE I.

Year.	Group.	Number of Calves.	Deaths from All Causes.		Cases of White Scour and Allied Infections.		Deaths from White Scour and Allied Infections.	
			Total.	Group Percentage.	Total	Group Percentage.	Total.	Group Percentage.
1937	A > 250 B.U.	67	8	11·9	5	7·4	0	0
	B < 250 B.U.	28	11	39·2	8	28·5	7	25
1938	A > 250 B.U.	83	4	4·8	9	10·8	1	1·2
	B < 250 B.U.	28	2	7·1	7	25·0	2	7·1
1937 and 1938 combined	A > 250 B.U.	150	12	8·0	14	9·3	1	0·66
	B < 250 B.U.	56	13	23·2	15	26·8	9	16·0
	χ^2		8·8555		10·27		20·95	
	P		0·003		0·0016		0·000008	

being very similar to the normal amount in milk on the third or fourth day, *i.e.*, a tenth or even a twentieth of the content present in the colostrum within a few hours after parturition.

Accurate records of 95 calves were obtained. The total number of deaths from all causes, the total number of cases of white scour and allied infections, and the total number of deaths due to such infections during the first two weeks of life are summarised in Table I. Since it is generally conceded that only extreme deprivation of vitamin A would predispose to certain infections the calves have been classified into two groups—Group A, those from cows whose colostrum contained over 250 B.U. of vitamin A per 100 ml. on the day of parturition and Group B, those from cows whose colostrum contained less than 250 B.U. per 100 ml. It may be added that in drawing a line at 250 B.U. only the results of the present experiments have been assessed and there is no evidence to be found in the literature to justify such a demarcation apart from the findings of the present paper. The results are recorded as Blue Units (Moore's) since in the calculation of International Units the carotene value is also assessed, and it is uncertain whether calves during the first few days of life are capable of utilising carotene by converting it into vitamin A.

The experiment was repeated during 1937-38. On this occasion the number of farms was limited to eight and the vitamin A content of the colostrum was estimated in samples obtained from cows calving from November to May, instead of February to May as in the previous experiment. This change of date allowed of collection of data from heifers which were due to calve in November and December. It was considered likely that these heifers calving soon after their return to stall-feeding would have much higher reserves of vitamin A than cows calving in the late spring. Altogether 110 cows were put on experiment and accurate records obtained of 111 calves. The vitamin A content of the colostrum on the day of parturition ranged from 2,590 to 86 B.U. per 100 ml. as compared with a range of 1,280 to 59 B.U. in 1937. It will be observed that the maximum has been increased by 100 per cent., but only seven out of the 110 cows exceeded 1,280 B.U. the maximum of 1937, and of those seven four were heifers calving very early. No very serious outbreaks of white scour or other similar diseases occurred in 1937-38, so that the death rate in the herds was comparatively low, but actually the number of cases, which eventually recovered, was slightly higher than in the 1937 experiment.

The data regarding the health of the calves are classified, as in the previous year, under two groups and summarised in Table I. In addition the results of both experiments have been combined and statistically examined by Colonel A. G. McKendrick and Dr. W. O. Kermack of the Royal College of Physicians Laboratory, Edinburgh, and the values for χ^2 and P are given in Table I.

Discussion of Results.

From Table I it is at once evident that the total death-rate, number of cases of *B. coli* infection, and deaths from such infections are much higher in calves of such cows whose colostrum has a lower vitamin A content than 250 B.U. per 100 ml. The values shown for P establish that the differences between the two groups A and B are highly significant. Especially is this true of the death-rate from white scour and similar infections—of ten deaths occurring from scour in the 206 calves under experiment nine belonged to Group B. Further, more than 25 per cent. of the calves in Group B became infected with either white scour, navel-ill or joint-ill, whereas only 9·3 per cent. of Group A was thus affected. In considering the results it must be borne in mind that over 70 of the cows on experiment in 1937 and all the cows in the 1938 experiment belonged to attested herds, so that from the point of view of management, dietary and hygiene the experiments were carried out under the most ideal conditions, yet 15 per cent. of the 206 calves became infected with *B. coli*-like organisms.

It appears highly probable, therefore, that if the cow's colostrum is very low in vitamin A the calf is more liable to infection. Moreover, if a cow's colostrum is high in vitamin A the calf has more chance of recovery if it happens to become infected by such organisms as are responsible for white scour, navel-ill and joint-ill. It must be emphasised, however, that it is quite unlikely that vitamin A in any quantity would affect the course of heavy infections of *B. coli* and allied organisms. Since the vitamin A content of the colostrum falls quickly in the successive days after parturition and remains practically constant at 100 or less B.U. per 100 ml. after the third or fourth day, it is obvious if the calf is to receive the maximum amount of vitamin A from its mother it is essential that it should be allowed to obtain the colostrum as soon after calving as possible.

It has already been mentioned that Stewart and McCallum (1938) demonstrated that one of the chief factors affecting the level of vitamin A in the colostrum of dairy cows was the length of the non-lactating period between successive calvings. It will readily be understood that lactating cows upon an artificial diet containing only an infinitesimal content of vitamin A will experience a heavy drain on their liver reserves. Therefore if the period between lactations is short these reserves have no chance of being restored and consequently the colostrum content must be low. The longer the lactation upon a diet of artificial food and the shorter the period between the end of lactation and the date of parturition, the smaller the vitamin A content of the colostrum. Experiments are now in progress designed to determine whether, during stall-feeding, a diet, rich enough in vitamin A to prevent lactating cows utilising their liver reserves, can maintain the level of vitamin A at a maximum

throughout the whole herd and thereby decrease the incidence of infection from such an organism as *B. coli*.

Summary.

Statistically significant results from experiments carried out on a calf population of 206 showed that calves born of mothers whose colostrum possessed a vitamin A content of less than 250 Blue Units (Moore's) per 100 ml. were more liable to infections such as white scour, navel-ill and joint-ill than were calves from mothers whose colostrum had a vitamin A content of more than 250 Blue Units per 100 ml.

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COTTONSEED POISONING OF PIGS

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The object of this paper is to describe an outbreak of fatal disease in a herd of pigs, the circumstances, symptoms and lesions of which were indicative of poisoning by the ingestion of cottonseed.

Literature.

Cottonseed and the meal and cakes derived from it have frequently been observed to be injurious to stock, pigs being particularly susceptible to its toxic action. In summarising the views regarding the toxity of cottonseed to different species of animals, Morrison (1936) states that investigations have shown that cattle are not affected by considerable amounts while pigs are seriously injured by similar quantities. Calves up to the age of four months are more susceptible that adult cattle. Horses and sheep are probably affected by smaller amounts than are cattle, but they will tolerate larger proportions than pigs. Steyn (1934), reviewing the literature on cottonseed poisoning, states that there seems to be a general consensus of opinion that pigs, especially when young, are very susceptible to cottonseed meal or cake poisoning, yet it may be fed to advantage to horses, cattle and sheep. Withers and Carruth (1916) record the death of pigs five to seven weeks after the commencement of the experimental feeding of a ration comprising one part of cottonseed meal and three parts of cornmeal. In another experiment six of nine pigs of varying weights died from five to eight weeks after the beginning of feeding a daily ration of 1 per cent. cottonseed meal and 3 per cent. cornmeal, based on the initial body weight ; all the smaller pigs died. Rommel and Vedder (1916) state that pigs are particularly susceptible to cottonseed poisoning, symptoms appearing at any time after three weeks of feeding, and death often occurring with little warning. Vald (1928) found that pigs fed on cottonseed meal in amounts varying from 10 to 30 per cent. of the total ration died after five to eleven weeks.

Several theories have been advanced with regard to the cause of the toxic action of cottonseed and its derivatives. These views have been reviewed by Steyn (1934) and are beyond the scope of this paper. It is probably true to say that the majority of workers now maintain that the toxic principle of cottonseed is gossypol, and much investigation on the pharmacology and experimental feeding of this substance has been carried out, chiefly in the United States.

There appears to be a relation between diet and susceptibility to cottonseed poisoning. Morrison (1936) states that recent investigations on the feeding of cottonseed to pigs indicate that good results are obtained when it is fed as part of the protein supplement, provided

that the ration does not contain more than 9 or 10 per cent. of cotton-seed meal and that protein of good quality, calcium, and vitamins are being fed. Pigs running on pasture appear to be less susceptible to poisoning than housed pigs. The rôle of diet in protecting against cottonseed poisoning is not clearly understood, but it has been observed by several workers that certain dietary constituents seem to diminish the toxity of cottonseed. Withers and Brewster (1913), McGowan and Crighton (1924), Gallup and Reder (1935), and Morrison (1936) record the action of iron salts in protecting against poisoning, it being suggested that a chemical reaction takes place between the iron and gossypol, probably resulting in the production of an insoluble iron-gossypol complex which is not digested. Robison (1934) reports that a protein supplement of good quality seems to have a specific effect in preventing cottonseed injury to pigs, and suggests that this may be due to the gossypol in the cotton-seed meal combining with the proteins in the digestive tract to form a harmless insoluble compound. Gallup and Reder (1935) state that a relatively high percentage of protein in the diet appeared to reduce the toxity of gossypol for rats. These authors conclude from the same series of experiments that calcium salts appear to be favourable to the detoxication of gossypol in the animal body, and suggest that a chemical reaction occurs between calcium and gossypol.

The symptoms described in cases of cottonseed poisoning vary somewhat in different species of animals. Rommel and Vedder (1916) state that acute and chronic types of poisoning occur in pigs, and give the following description of the acute form: pigs are attacked with little warning and may be dead before any indications of disease are noticed. The attack is sudden and sharp, the pig experiencing extreme shortness of breath and suffering intense pain. Vomiting was observed in a mature sow, no other symptoms being seen.

The lesions of cottonseed poisoning are as a whole similar in the different species of farm and laboratory animals affected. The following *post-mortem* findings are recorded by Rommel and Vedder (1916) in pigs that have died from the effects of cottonseed poisoning: large quantities of fluid in the abdominal and thoracic cavities and in the pericardial sac; congestion of the liver, spleen, kidneys, and small intestines; erosion of the gastric mucosa in some cases; marked œdema of the lungs, especially in pigs which have died from sudden acute attacks; enlarged heart. Steyn (1934), summarising the observations of several workers, states that the following lesions are found in acute cases of poisoning: swelling of the gastro-intestinal mucosa, blood-stained gastro-intestinal contents, hydroperitoneum, hydrothorax, hydropericardium, "blood-stained urine," sub-epicardial and sub-endocardial hæmorrhages with degeneration of the myocardium, acute hæmorrhagic parenchymatous nephritis, liver markedly swollen and very soft, and ulcerative keratitis. Schwartze and Alsberg (1924b) describe œdema of the lungs which may be hæmorrhagic,

cardiac hypertrophy, and effusion into the serous cavities in experimental poisoning of cats and rabbits. They state that administration of gossypol causes œdema of the lungs in rabbits, guinea-pigs, cats, rats, and mice. Fatal œdema in these animals is hæmorrhagic and a blood-stained exudate may appear at the nose. Withers and Carruth (1916) observed excess of fluid in the abdominal cavity, enteritis, congestion of the liver and kidneys, and œdema and congestion of the lungs in rabbits experimentally fed on gossypol.

Cottonseed Poisoning in Nyasaland.

The Affected Herd.

The cases of cottonseed poisoning described in this paper occurred in a herd of about fifty grade Large Black pigs belonging to a mission in the Southern Province of Nyasaland. The herd, which had been maintained for about fifteen years, was kept solely for the purpose of supplying the European staff of the mission with pork, it being the practice to slaughter the young pigs at the ages of six to eight months. The herd was in the charge of a fairly intelligent native pig-boy, whose account of the history of the cases is probably reliable. This native was under European supervision, it being the custom for a member of the mission staff to look at the herd each evening.

In view of the apparent relation between cottonseed poisoning and nutritional deficiency, the system of management practised at the mission will be described in some detail. The feeding and the conditions under which the pigs were kept were unsuited to the proper maintenance of improved breeds, and the pigs were poor in type and condition. They were housed in a brickwork building containing seven adjoining sties, each consisting of a dark and ill-ventilated pen and a small open run with a cement floor. The pigs were always confined in the sties, and were not allowed free range on pasture. At the time the poisoning occurred three sties contained sows with litters of suckling piglets, the boar in the fourth, and four brood sows in a fifth. The sty which will be referred to as number 6 contained nine young pigs aged about four months, and number 7 two litters, comprising fourteen pigs, aged about five and six months respectively.

The pigs were fed principally on maize. The main portion of the ration consisted of a mixture of cooked wholemeal maize flour and cooked native beans, a *Phaseolus vulgaris*, which had been left over the previous day from the native schools attached to the mission. To about three parts of this mixture one part of raw kibbled maize was added and the whole made into a sloppy mash with water. The feed was mixed and prepared by the pig-boy so that these proportions are only approximately correct. A definite amount of this mixture was not fed daily, the pigs being given as much as they would clear up. The proportion of beans in the diet probably varied but was not large. The ration issued in the mission schools contained approximately one part of beans to three of maize flour. Beans are regarded

as a relish by the natives, maize being the staple foodstuff, and it is probable that as a rule comparatively little beans were left over and sent to the piggery; the mixture was examined twice and on each occasion the quantity of beans was in fact found to be small. In addition freshly cut elephant grass (*Pennisetum purpureum*) was fed daily. No protein-rich supplement or minerals were fed. It is evident that the pigs were being fed on a diet deficient in protein and minerals, particularly iron and calcium. It was noticed that the lime mortar pointing of the brickwork had been gnawed away to a height of about three feet in all the sties, a possible indication of the mineral deficiency in the herd. Deficiency of vitamins A and D would be prevented by the feeding of elephant grass and by exposure of the pigs to sunlight.

History and Investigation of the Outbreak.

On January 18th, 1938, a report was received from the mission stating that eight pigs had died since January 12th, five during the past three days. The mission was visited early on the 19th, when it was found that two further pigs had died, one in sty 7 during the night and one in sty 6 a few minutes previously. All ten deaths had occurred among the young pigs in sties 6 and 7, five of the first eight pigs to die having been in sty 6. There had been no deaths among the adult pigs or the suckling piglets. One sow was reported to have vomited repeatedly two or three days previously; this had been the only unusual symptom noticed in any of the pigs in sties 1 to 5.

The pigs in all the sties were examined, the young pigs in the affected groups being examined individually and temperatured. All appeared normal and no evidence of disease was seen. During the following four hours, two pigs died in sty 7, one about two hours after the other. Both deaths were extremely sudden, the pigs having shown no evident symptoms until less than an hour before they died.

Post-mortem examinations were made on the four pigs which died on January 19th. Heart-blood and thoracic fluid were preserved in equal parts of 50 per cent. glycerin, in order to determine by inoculation whether the deaths were due to East African swine fever. Smears were prepared from the heart-blood, mesenteric lymph glands, spleen, and pulmonary exudate of all four pigs. Examination of these smears, stained with 1 per cent. methylene blue, Gram's method, and Giemsa stain, did not show the presence of any pathogenic organism. Cultures made from the heart-blood and spleen pulp were sterile.

At the laboratory two pigs, purchased in another district, were inoculated with material collected *post-mortem*. The first was inoculated subcutaneously with 10 c.c. of a mixed sample of preserved blood from the second and third pigs to die. The second was inoculated subcutaneously with 10 c.c. of heart-blood withdrawn from the fourth pig about an hour after death. These two pigs were

kept under observation, being temperatured daily; both remained normal in health and showed no unusual symptoms.

The following pertinent information was obtained. No additions to the herd had been made during the previous nine months. No infectious disease had occurred in the herd for at least four years. There was no infectious disease of pigs in the native villages surrounding the mission. About four weeks before the first death occurred, cottonseed had been introduced into the ration of the pigs. Raw cottonseed is fed to cattle without evident ill-effect by many owners living near ginneries in the cotton-growing districts of Nyasaland, and it had been assumed that cottonseed could safely be fed to pigs. The cottonseed had been lightly cooked and fed in the proportion of about one part to three of the ration usually fed. This mixture was consumed readily enough, but it apparently was not relished and after about two weeks the cottonseed had been discontinued. Excepting the cottonseed, no substance which might have caused fatal poisoning had been given to the pigs.

During the night of the 19th a further death occurred in sty 6, and on the 20th one of the remaining eight pigs in sty 7 died. Since then there have been no deaths in the herd. In addition to the sow mentioned above, two sows were reported to have gone off their feed, vomited, and developed mild diarrhœa during the week following my visit. The entire herd was examined three weeks later. All the remaining pigs appeared completely normal and healthy, and have remained so since then.

Symptoms.

The pig-boy gave the following description of the symptoms shown by the pigs he had seen to be ill; he was not clear about how many of the ten pigs he had actually seen showing symptoms and dying. All deaths had been very sudden. The pigs had been ill for a very short period, "less than an hour" according to the pig-boy, and to have shown increasing difficulty in breathing, until they died discharging red foam from the nostrils. No pigs which had shown symptoms of respiratory distress had recovered. No other sign of disease had been seen.

The symptoms described by the pig-boy resemble those shown by the two pigs seen to die on January 19th. These were first noticed to be standing apart from the other pigs with the head extended, the mouth open, the ears and tail drooping, and the fore-limbs set wide apart. Both showed great respiratory distress, the breathing being deep and accelerated with a grunting expiration. Examination showed cyanosis of all the visible mucous membranes, and typical symptoms of asphyxia. Dyspnœa increased rapidly, the breathing becoming stertorous than gasping. Within half an hour of symptoms first being noticed, the pigs developed convulsions lasting two or three minutes and died, discharging a quantity of sanious frothy fluid from the nostrils.

Lesions.

The findings in the four pigs post-mortemed were uniform, except for the degree of pathological changes present in individual pigs, and the following description of lesions is based on all four *post-mortem* examinations. Three of the post-mortems were carried out within an hour, and the fourth within twelve hours of death.

The carcase condition was good. The integument appeared normal except for cyanosis of the hairless parts (abdomen, inside the thighs, and axillæ). The visible mucous membranes were cyanotic but otherwise normal. A sanious frothy discharge was present around the nostrils. There was slight subcutaneous œdema. The flesh appeared normal.

The peritoneal cavity contained about 150 c.c. to 250 c.c. of clear straw-coloured serous fluid. The serous membranes were normal. The spleen was normal in size, and in consistency of the pulp. The œsophagus appeared normal. The stomach was filled with recently ingested food, mixed with yellowish-grey mucus. There was a diffuse swelling and catarrhal, slightly hæmorrhagic inflammation of the gastric mucosa, which was most marked towards the pylorus ; slight submucous œdema was seen. Catarrhal and patchy hæmorrhagic inflammation was present throughout the small intestines ; the contents were semi-liquid and greenish-brown in colour ; the lymph-nodes were swollen and slightly hæmorrhagic. A few *Ascaris lumbricoides* were found. The mesenteric lymph glands were uniformly enlarged and congested, and were dark reddish-grey on section. There was slight catarrh of the large intestine and rectum. Fairly heavy infections of *Oesophagostomum dentatum* and *Trichuris suis* were found. The fæces were soft but not liquid, and contained some mucus. The liver was enlarged and congested, with the capsule distended ; the cut surface was deep reddish-purple in colour, and exuded a considerable quantity of dark blood. The gall-bladder and contents appeared normal. The kidneys were swollen, congested, and dark red in colour: the capsules were distended and stripped easily; there were numerous small subcapsular and intracortical hæmorrhages, on section dark blood exuded from the cut surface. The ureters, bladder, urine and genital organs all appeared normal.

The pleural cavity contained about 70 to 120 c.c. of clear straw-coloured serous exudate. The serous membranes appeared normal. The larynx was normal. The trachea and upper respiratory passages were filled with a large quantity of frothy blood-stained fluid ; the mucous membrane of the trachea was slightly congested. There was very pronounced congestion and œdema throughout the lungs, which were brick-red to purple in colour ; the surface of the lungs was bulging and firm, with the lobulation distinct. On section the edges were everted ; the colour of the cut surface was dark-red to purple ; the inter-lobular septa were distinct. A large quantity of blood-stained frothy fluid flowed from the cut surface. The bronchi were filled with sanious frothy fluid. The pericardial sac contained

10 to 15 c.c. of clear serous fluid. The heart was enlarged and flabby, the left ventricle particularly so ; the myocardium was pale and soft ; the epicardium and endocardium appeared normal and no hæmorrhages were seen.

Discussion.

In the absence of definite proof, the diagnosis of cottonseed poisoning as the cause of the outbreak recorded in this paper depends on the exclusion of the possibility that deaths were due to specific disease, the history of feeding cottonseed and the "incubation period," the sudden onset and equally sudden cessation of deaths corresponding to the commencement and stopping of feeding cottonseed, the ages of the affected pigs, the symptoms, and the lesions.

The only infectious disease of pigs occurring in East and South Africa, with which this outbreak might be confused, is that called East African swine fever by Montgomery (1921) and East African virus disease in Pigs by Steyn (1932). In some respects this disease resembles closely the mission outbreak. The disease is almost invariably acute or peracute, the onset being sudden and the course rapid, death being preceded by convulsions. Montgomery (1921) states that epizootics are sometimes ushered in by apparently sudden deaths. The *post-mortem* findings seen at the mission were very similar to those described in E.A. swine fever. The following characteristics of E.A. swine fever, recorded by Montgomery (1921). Steyn (1928), Steyn (1932), and Walker (1933) differentiate the disease from the mission outbreak, and give indisputable proof that the deaths were not due to E.A. swine fever. The disease is always febrile, and incoordination or paralysis of the hind-limbs is usually seen. All classes of pigs are equally susceptible, no age, breed, or sex possessing any resistance. The mortality in natural and experimental infections approximates 100 per cent.; in some outbreaks all pigs in a district have died. Blood collected from pigs which have died of the disease is invariably virulent, and subcutaneous inoculation into pigs always produces infection. Susceptible pigs in contact with clinically reacting animals become infected, and infection is carried from sty to sty by the attendant. Tumor splenis, and subepicardial and subendocardial hæmorrhages, characteristics of the disease, were not found in the mission outbreak.

In the outbreak here recorded eight adult and twenty-three young pigs, aged four to six months, were fed on cottonseed to the amount of about 25 per cent. of the total ration for approximately two weeks. During the fifth week after the beginning of feeding, seven of nine pigs aged four months, and seven of fourteen pigs aged five to six months died. Generally speaking the pigs aged four months died earlier than those in the older group. Of the eight adult pigs, one vomited and two showed transient vomiting, mild diarrhœa, and anorexia; no other symptoms were observed. It was evident that the young pigs were particularly affected by the injurious agent,

which fact agrees with the common observation that, in cottonseed poisoning, young animals are more susceptible than older ones. The fact that some of the pigs showed no symptoms was presumably due to individual variation in susceptibility. Schwartze and Alsberg (1924a) state that differences in susceptibility to cottonseed poisoning between animals of the same age have been recorded by several workers. The syndromes seen in the mission outbreak agree with the brief account of acute cottonseed poisoning given by Rommel and Vedder (1916), and the *post-mortem* findings resemble closely the recorded descriptions of lesions caused by cottonseed poisoning. From the collected evidence summarised above the assumption appears to be warranted that poisoning by cottonseed was responsible for the symptoms and deaths in the mission outbreak.

Certain points of interest arise out of the observations made in the investigation of this outbreak.

Raw cottonseed is not commonly fed to stock, and feeding is presumably restricted to cotton growing areas in which oil-mills, purchasing seed and manufacturing cottonseed derivatives, do not exist. In comparison the quantity of meal and cakes fed to stock is enormous and, as is to be expected, most accounts of cottonseed injury to livestock describe poisoning by the derivatives. The observations recorded in this paper show that cases of poisoning showing symptoms and lesions essentially similar to those caused by cakes and meal are produced by the feeding of unaltered cottonseed. This indicates that the injurious agent is present in the raw seed and is not, as has been suggested it may sometimes be, a toxic substance contained in the derivatives, such as impurities in the solvents used to extract the oil, or products of decomposition of the cakes.

Considering next the question of the form of poisoning encountered in the mission outbreak. Excepting the mild symptoms seen in the three sows, all cases were of the acute rapidly fatal type. Compared with the amounts quoted above as having caused fatal poisoning to pigs, the quantity of cottonseed received by the mission pigs was not large, and it is tentatively suggested that either or both of two factors may have assisted in the causation of the very severe effects produced by the feeding, during a short period, of a comparatively small amount of seed. First, the cottonseed fed at the mission may have been a particularly toxic variety. Schwartze and Alsberg (1924a) and Morrison (1936) record that a regional variation in the toxicity of different varieties of cottonseed does occur, and it is possible, although there is no evidence to support the hypothesis, that a highly toxic type of seed was involved in this outbreak. The second factor, which would appear to be the more probable in view of the observations quoted earlier in the paper, is that by reason of a nutritional deficiency, and more especially a deficiency of protein, iron, and calcium, the mission herd was as a whole highly susceptible to cottonseed poisoning. It is suggested that the observation that the severe effects recorded in this paper may be produced by the feeding of

cottonseed during two weeks to a herd of pigs on a diet deficient in these foods, supports the view that there is a relation between susceptibility to cottonseed poisoning and nutritional deficiency.

Summary.

An outbreak of cottonseed poisoning in a herd of pigs in the Southern Province of Nyasaland is described.

Cottonseed to the amount of about 25 per cent. of the total ration, fed for two weeks, caused peracute fatal poisoning during the fifth week after the commencement of feeding, of fourteen out of a total of twenty-three pigs aged four to six months. Generally speaking, the younger pigs died earlier than the older ones. Of eight adult pigs one vomited and two showed anorexia, vomiting and diarrhœa.

The symptoms and lesions observed in the outbreak are described.

It is concluded that cases of poisoning essentially similar to those caused by cottonseed cakes and meal are produced by unaltered cottonseed, and that the toxic agent is therefore present in the raw seed.

The ration fed to the affected herd was deficient in protein and minerals. It is suggested that the very severe effects, produced by a comparatively small amount of cottonseed, support the view that there exists a relation between susceptibility to cottonseed poisoning and nutritional deficiency, particularly a deficiency of protein, iron and calcium.

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THE TOXICITY OF INTESTINAL FILTRATES FROM LAMBS DEAD OF OVEREATING.*

By I. E. Newsom and Frank Thorp.
From Colorado Experiment Station.

A comparison of overeating as described by this station and enterotoxaemia as reported by Bennetts in Western Australia reveals that both diseases affect lambs in good condition and those on rich food. Both are acute diseases and frequently result in death during the night without observed symptoms. Either may show convulsions and both are associated with rapid *post-mortem* decomposition (pulpy kidney).

Those signs which are regarded as charactertistic of overeating, such as cerebral disturbance (throwing the head back, pushing against the fence), the presence of blotchy haemorrhages under the peritoneum, in the abdominal muscles and in the diaphragm, the presence of quantities of plasma in the pericardial sac, and finally the finding of from 2 to 6 per cent. of sugar in the urine are not described by the Australian investigators (the Reference here is to Oxter and Bennetts, 1932).

In spite of these decrepancies it seemed that the toxicity of intestinal filtrates taken from lambs dead of overeating should be determined. Accordingly the work was started in 1932 and has been continued down to the present time (reference here to previous articles).

This present paper is a brief summary of one phase of the broader problem which will be reported in detail at a later date.

The small intestines were selected from the lambs as soon as possible after death, stripped of their contents, an equal amount of 0·85 per cent. salt solution added and the material filtered through several layers of gauze. This was then centrifuged at about 2,500 r.p.m. for an hour, the supernatant fluid drawn off and passed through a clay filter. After sterility had been checked it was inoculated intramuscularly into the thighs of guinea-pigs, usually in 4 c.c. doses, two on each side. Berkefeld and Mandler filters were used during the first two seasons 1932-34. In 1934-35 the material was passed through Chamberland and then through Seitz filters.

Beginning in 1935 the supernatant fluid after centrifugation was filtered three times through filter paper, first through four, eight and then twelve layers moistened with water. It was then injected intravenously into rabbits in from 0·5 to 1·2 c.c. amounts according to size, and if not toxic it was discarded. If toxic it was sterilised by passage through a Seitz filter.

During the first two years guinea-pigs were injected intramuscularly. During the rest of the time rabbits were inoculated intravenously. Mice were used sometimes but only for check tests.

Results with Laboratory Animals.

Over a period of six years 343 filtrates were thus tested, 256 being from lambs dead of overeating and 87 from those dead of other diseases. Of the 256 from cases of overeating, 115 (45 per cent.) were toxic either for guinea-pigs or rabbits, while of the 87 dead from other diseases only two were injurious to these laboratory animals. One of these was taken from a lamb dead of coccidiosis and one from an animal dead of a

* From the *Jour. Am. Vet. Med. Assocn.*, September, 1938.

ruptured bladder. The authors think that on looking back over the records of these cases in the light of negative results of subsequent years, there can be little doubt of their authenticity. Both were diseases that were unlikely to be mistaken and in both instances so much work was done with the filtrates that there can be no question of the toxicity.

Results with Sheep.

During the first season 1932-33 eight of the nine toxic filtrates were injected into the axillae of sheep in 25 c.c. doses equally divided between the two sides. Six of the injected animals died within 24 hours. The two others survived after showing swelling and lameness. The lesions of the dead sheep consisted of large accumulations of sterile clear plasma, non-caseous and non-haemorrhagic. In some instances extending downward to the knee, forward to the throat and backward to the lower flank. The remaining filtrate was not used because the amount was too small to justify injecting it.

In 1933-34 five filtrates were found toxic for guinea-pigs and of these four were injected into the axillae of sheep in 25 c.c. doses. Three of the sheep died with lesions as in the previous year and one survived after being desperately ill for 24 hours. One of these filtrates which killed the sheep was from a lamb dead of coccidiosis. In two instances 10 c.c. doses failed to kill sheep where 25 c.c. doses had done so.

In 1935-36 only two filtrates were tested on sheep. One of these killed sheep in doses of 25, 12 and 5 c.c. and the other failed to kill in 15 c.c. dosage.

In 1937-38 six filtrates were tested on sheep. One of them killed in 30 c.c. dosage but failed to kill in amounts of 12 and 5 c.c. and another in 65 c.c. Two others produced toxic symptoms after the administration of 50 c.c. but did not destroy.

Special attention was given to the urine in all cases but in most of them the bladder was empty. In three instances sugar was present. In one of the 1932-33 series, 0·42 per cent. sugar was present, and in another just a trace. One of the 1933-34 animals showed 1·43 per cent. sugar. Urine was obtained from the two sheep in 1937-38, and while both showed albumen neither gave a test for sugar.

Only one animal showed the blotchy haemorrhages considered typical of overeating, that being one of the 1937-38 lot.

Nature of the Toxin.

In the earlier years each toxic filtrate was heated in a water bath of 60° C. for 30 minutes and then injected into guinea-pigs in the same dose that had proved fatal. In every instance the guinea-pig survived, indicating that the heating had destroyed the toxin. In the later years routine heating was frequently neglected, but in those cases where the effect of heating was tested the results were the same.

In 1934-35 the five toxic filtrates were incubated with antitoxins obtained from the Wellcome Laboratories, London, to determine the neutralising effect of these serums. They were labelled "B. welchii," "B. paludis," "B. ovitoxicus" and "lamb dysentery." While there was some variation in the tests conducted in general 0·5 c.c. of the filtrate was incubated at 37° C. with 0·5 c.c. of the antiserum for one hour and the whole was then injected intravenously into a rabbit. In every trial the *B. ovitoxicus* (Wilsdon's type D) and lamb dysentery (Wilsdon's type

B) neutralised the filtrate, while the *B. welchii* (Wilsdon's type A) and *B. paludis* (Wilsdon's type C) failed to do so.

These filtrates failed to cause symptoms in rabbits when given by the mouth in 10 c.c. doses.

In 1935-36 eight of the filtrates were tested only against antiserums for *B. welchii, B. paludis* and *B. ovitoxicus,* the lamb dysentery serum having become contaminated in the meantime. All eight were completely neutralised by the *B. ovitoxicus* serum but remained toxic in the presence of the two others.

Discussion.

Bennetts found 14 toxic filtrates out of 16 examined (88 per cent.), which was higher than in any season reported here. In the earlier years, however, the manipulation necessary to obtain sterility may have been responsible for some of the weaker filtrates losing their toxicity. In some years also the filtrates were held a considerable time before being tested owing to a shortage of appropriate test animals. In the 1937-38 season, when most of these difficulties were overcome, the percentage of toxicity ran to 70, thus more nearly approaching Bennetts' figures.

Bennetts found, as in the present study, that his filtrates were neutralised by both the lamb dysentery and *ovitoxicus* antiserums and were not rendered harmless by antitoxins made from *B. welchii* and *B. paludis.* If it cannot be assumed that overeating is due to the multiplication of *B. ovitoxicus* (*Cl. welchii* type D) and the elaboration of its toxin in the intestinal tract, then it seems that a reasonable explanation for the development of the disease is that the consumption of a large amount of concentrate tends to develop conditions favourable to the growth of that organism. As a practical procedure the disease has been controlled during the past 30 years by reducing the grain ration and increasing the roughage content of the diet.

While the toxin is undoubtedly injurious to laboratory animals as well as to sheep when injected parenterally, the authors have so far been unable to confirm Bennetts' finding and demonstrate its toxicity when given by the mouth. It can therefore only be assumed that it so injuries the epithelium of the small intestine that it is absorbed into the circulation.

Summary.

Forty-five per cent. of intestinal filtrates from 257 lambs dead of overeating were shown to be toxic for laboratory animals.

Of 87 similar filtrates from lambs dead of other diseases only two were toxic, one from a lamb dead of coccidiosis and one from a case of ruptured bladder.

Twelve out of 20 of the filtrates proved fatal to sheep when injected in amounts varying from 5 to 50 c.c.

Heating the filtrates to 60° C. for 30 minutes rendered them harmless.

The toxin was neutralised by antiserums made from the lamb dysentery bacillus and from *B. ovitoxicus,* but not those made from *Cl. welchii* or *B. paludis.*

While overeating seems to differ from enterotoxaemia on the basis of symptoms and lesions, the intestinal contents of lambs dead of the two diseases contain toxic substances that seem to be identical.

REVIEWS

Veterinary Obstetrics (Equine and Bovine). By Dr. Franz Benesch, Professor of Obstetrics and Inspector of the Obstetrical Clinic in the Veterinary High School, Vienna. English Translation. Edited by John G. Wright, F.R.C.V.S., Professor of Surgery at the Royal Veterinary College, London. London: Baillière, Tindall and Cox, 1938. Price 12s. 6d.

Professor Benesch's text book in English will prove a valuable addition to the literature on veterinary obstetrics, as may be inferred from the fact that it had previously been translated into Spanish and Italian. The translation and publication of the present edition were supervised by Professor J. G. Wright, of the Royal Veterinary College, and the text is very clear and leaves no trace of the original language.

The 85 Figures, mostly full page, are a strong feature of the work. They are obviously reproduced from careful drawings made to show the exact relationship of the foal or calf to the axis of the pelvis in the most frequent mal-positions.

The opening chapter describes in a general way the forces that come into play in normal parturition and the factors which give rise to dystocia and how these can be countered.

The second chapter is devoted to epidural anaesthesia and in it the method of performing this delicate operation is described and illustrated by pictures which show the exact point for the insertion of the needle in both the cow and the mare.

As the text explains, by the use of epidural anaesthesia abdominal counter-pressure becomes removed and space is thus procured for manipulation and the amount of force required is reduced. An important fact is that it can be employed by the obstetrician working without assistance under farm conditions.

Presence at difficult parturitions as a witness or helper is the ideal way by which a student can be trained in obstetrics, but in the existing circumstances that is not practicable, and one can conceive of no more repellent task for a teacher than the duty of teaching obstetrics as an abstract science. Dr. Benesch's work will, in that respect, be a boon to both teacher and students.

Fröhner-Zwick Compendium of Special Pathology and Therapeutics for Veterinary Surgeons. Edited by Dr. David Wirth, Professor in the Veterinary High School in Vienna, and Dr. Wilhelm Zwick, DR.MED.VET., Professor in the University of Giessen. Fifth Revised and Enlarged Edition, 1938. Ferdinand Enke, Stuttgart. Price R.M. 27.

In the first place the book is intended for students, but veterinary surgeons in practice will also find it useful for quick consultation when that is necessary regarding any important question.

The first three editions of this work were published by Professor Fröhner and in the new Fourth Edition the first part has been revised by Professor Dr. E. Fröhner, Berlin, and the second part by Professor Dr. William Zwick, Giessen. The first part occupies 161 pages and the second 193 pages.

The work in both parts has the ring of experience and it is sufficiently

full for the purpose of either practitioner or student. It is not burdened with referencess and the language is very clear.

The credit which had been deservedly acquired by the First Edition is fully maintained. The paper and printing are very good.

Virus and Virus Diseases in Man, Animals and Plants. By Dr. Gustav Seiffert. Published by Theodor Steinkopff, Dresden and Leipzig, 1938. Price: Unbound 16 RM, Bound 17 RM.

The work, including the Index, extends to 216 pages and it has seven Figures in the text. The first or general part extends to 62 pages and deals with the history and nature of the virus diseases, the size of the viruses and method of measurement, filtrability, pathogenesis, natural and acquired immunity to virus diseases, protective inoculation and epidemiology. The remaining or special part of the work deals with all the diseases that have established a claim to the title Virus Disease in man and animals, and references are given to the most important articles that have been published regarding each disease.

A special chapter deals with the diseases caused by virus-like organisms, including contagious bovine pleuro-pneumonia, agalactia, rickettsia, bartonella and bacteriophage.

The methods of examining the viruses are fully dealt with, including the methods of filtration, the varieties of filters and their use in determining the size.

Tuberculosis and Meat Inspection (Fleischhygiene). By Dr. med. vet. Karl Nieberle, Professor and Director of the Veterinary Pathological Institute of the University, Leipzig. In collaboration with Professor Dr. A. Meyn. Published by Gustav Fischer, Jena, 1938. Price, bound RM 7.20.

The opening chapter describes the nature of tuberculous disease, and concludes that the lesions are of an inflammatory nature, and that the inflammation serves for the protection of the body.

The second chapter, which is headed Origin and Development of Tuberculous Disease, opens with the statement "Tuberculous disease in man and animals begins with the primary lesion." It is declared that this short sentence marks one of the greatest scientific achievements in researches regarding tuberculosis during the last 20 years, and at the same time a discovery of the greatest practical importance.

That tubercle bacilli arrive in the body in various ways was always obvious—with the food, be inhaled with the air, or from the diseased mother through the blood in the umbilical vein of the foetus (congenital tuberculosis). The author asks, do these general possibilities enable one in practice to recognise the way the bacteria have actually entered any particular case? Can one with certainty determine what is the practical importance of these recognised possible ways? The answer to these important questions was formerly in the negative. It was held, for example, that the bacilli arriving in the intestine with the food did not encounter any reaction of the intestinal mucous membrane and were able to pass through the mesenteric glands and by way of the thoracic duct obtain admission to the circulating blood and thus into the lungs, to be arrested there and be responsible for the first lesions. It was thus declared to be impossible to determine what was the seat of the

first tuberculous lesions by the route of infection—in other words, one had to renounce the possibility of being able to recognise the exact route of infection in tuberculosis. In the author's opinion, however, that is no longer the case.

The present day doctrine of the primary tuberculous lesion asserts that the tubercle bacilli remain where they for the first time penetrated in an animal previously free from the disease, there leave their tracks, both in the reception organ at the place of their penetration and also in the regional lymph nodes of the organ. Thus, if we wish in any case to arrive at the way of infection of a tuberculous attack we must recognise only the primary lesion and its situation. "In other words, the problem of the route of infection in tuberculous disease is now, for the first time, anatomically soluble."

How did this doctrine of the primary lesions arise, and on what is it founded? The author's reply is that it did not arise from theoretical considerations but from the practical observation on tuberculous human patients. "Parrot and Kuss long ago at the *post-mortem* of children dead from various diseases observed that as a complication quite definite tuberculous lesions were not seldom recognisable in the lungs. Ghon, at the German University in Prague, by systematic examinations, could confirm the fact that children who had not died from tuberculosis frequently had in the lungs tuberculous lesions in the form of isolated caseous centres, and with great regularity the lymphatic glands belonging to that part of the lung also showed tuberculous lesions. Since other tuberculous lesions might be entirely absent Ghon concluded that these lesions actually formed the starting-point of the tuberculous disease and that here was the primary tuberculous centre. K. E. Ranke also studied these primary pulmonary centres, and since as a rule lungs and pulmonary lymph nodes were simultaneously affected he introduced for them the designation "primary-complex."

The remainder of the book may be described as an attempt to write a new account of the situation, origin, structure and appearance of tuberculous lesions in the domesticated animals, in conformity with this new theory.

According to the author, the doctrine of the primary lesions is also confirmed by statistical and experimental evidence. "Clearly the primary lesion actually represents the beginning of every case of tuberculosis and it remains recognisable as such in all the later stages of tuberculous disease, and therefore must in practically every case remain recognisable." It is stated that great numbers of investigations in this direction carried out with regard to the human subject have shown that in practically every case the primary lesion is recognisable as such and that in about 90 per cent. of the cases it is situated in the lungs and in about 10 per cent. elsewhere.

It is admitted that this new doctrine regarding the primary lesions must be supported by experiment and in the author's opinion that has been abundantly provided by the various experiments which have been carried out on animals and in the diagnosis of cases of tuberculosis by the inoculation of guinea-pigs and rabbits employed almost daily (but no references are given).

"The doctrine of the primary lesions is in any case no longer a problem, it is a firmly founded fact."

The chief purpose of this review has now been achieved—it was

to make accessible to English readers an authoritative account of what is meant by the theory of the "Primary Lesion."

But this Journal also claims the right to express an opinion on the validity of this theory and the inferences in regard to meat inspection which the author has founded on it.

Dr. Nieberle has designated the belief in the primary lesions theory a doctrine, but since all knowledge previously acquired regarding tuberculous lesions must be renounced if it is not in harmony with the doctrine, "creed" would appear to be a better name.

The question at issue concerns meat inspection, of which the main purpose is to detect and condemn as dangerous for human food the carcases of animals in which tubercle bacilli have at the time of slaughter been disseminated throughout the body in the blood stream.

The regulations which have governed meat inspection in Berlin and throughout Germany for 50 years have that object, and they were based on the view that the object would be secured if cases of so-called "acute generalised tuberculosis" could be detected and destroyed. Throughout Europe similar rules are enforced.

In this matter Dr. Nieberle's opinions are heterodox. He has satisfied himself, and wishes to persuade others to believe, that under the existing regulations the flesh of tuberculous animals may contain tubercle bacilli and escape detection. He has so far failed to provide any figures to prove that opinion, and has ignored facts that are opposed to it. Readers of his work will find that it is almost destitute of references, but that may be in accordance with his anathema against researches carried out before the discovery of the doctrine of the primary lesions.

One of the few references is to Henschel, who was the author of a valuable monograph on the subject of tuberculosis in animals. He was appointed for one year by Herr von Ostertag to study tuberculosis in the animals killed in the city abattoir in Berlin. His report showed that during the year 1909 the number of oxen, bulls, and cows, taken together, was 130,524, of which 45,339 were infected, and there was only one case of acute generalised tuberculosis.

The author also refers to Weigert in connection with acute generalised tuberculosis in man, but omits to state that that distinguished pathologist was the first to discover and define the exact nature of that form of the disease and show how rare it is in human consumption.

It is an unproved assumption that this acute, rapidly fatal form of tuberculosis is other than rare in cattle (or man) in any part of the world. It is therefore probable that the majority of such cases never reach a slaughterhouse, and those that do could be detected by the use of a thermometer.

In the text there are 38 low-power micro-photographs of stained sections, and at the end a number of coloured plates, but they do nothing to confirm the new doctrine.

We greatly regret that we cannot recommend the book as a guide to the study of tuberculosis in the domesticated animals, but it may be said to contain the only authoritative account of the "doctrine of the primary lesions."

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